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Thesis

VITAMINS

Submitted by

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TABLE OF CONTENTS

"VITAMINS"

OUTLINE

"VITAMINS"

CHAPTER I

DISCOVERY AND NOMENCLATURE OF VITAMINS

(a) EVIDENCE FROM OBSERVATION UPON DISEASE

(b) EVIDENCE FROM EXPERIMENTS UPON NORMAL NUTRITION

(c) TERMINOLOGY OR NOMENCLATURE

(1) OF VITAMIN A

(2) OF VITAMIN B

(3) OF VITAMIN C

(4) OF VITAMIN D

(5) OF VITAMIN E

(6) OF VITAMIN F AND G OR B_1 OR B_2 OR PP

CHAPTER II

VITAMINS IN PLANTS

(a) SEPARATION OF THE VITAMIN- LIKE "BIOS"

(b) GREENESS OF VITAMIN A IN PLANT TISSUE

CHAPTER III

VITAMINS IN ANIMALS

(a) INSECTS

(b) RATS AND VITAMIN E

CHAPTER IV

QUANTITATIVE DIFFERENTIATION OF VITAMIN A AND D

(a) EXPERIMENTAL METHOD

(b) AVERAGE OF BODY WEIGHT AND BODY CALCIUM IN TEST ANIMALS RECEIVING LIMITED ALLOWANCES OF VITAMIN A CONTAINING FOOD WITHOUT AND WITH ULTRA-VIOLET IRRADIATION

CHAPTER VII (CONT)

(e) APPENDIX TO CHAPTER VII

EFFECT OF HEAT AND PRESERVATION PROCESSES ON VITAMINS

CHAPTER VIII

SUMMARY

Chapter I

Discovery and Nomenclature of Vitamins

"Until (1) recent years, the discussion of foods and nutrition from the chemical point of view was hampered by the embarrassing fact that all attempts at prolonged feeding upon artificial mixtures containing the substances known to be necessary in nutrition had ended in failure. Whether nutritive failure resulted from the need of other substances than those known as essential, or from faulty selection of quantitative combination of the nutrients entering into the artificial food mixture remained obscure until the work of Hopkins (2) in England, and of Osborne and Mendel (3) and McCollum (4) and Davis in this country made it clear that a natural food supply furnishes, and a normal nutrition requires other substances in addition to proteins, fats, carbohydrates, water and salts. With this fact convincingly established, it is easier to see that it was foreshadowed by many earlier observations than it is to say definitely when and by whom the existence now known as vitamins was discovered. Our present "vitamin theory", or point of view, in regard to this branch of food chemistry, is rather the product of development than of any isolated discovery, and much of this development antedates the introduction of the word vitamin."

(1) (2) (3) (4) Sherman H. C. and Smith S. L. - "The Vitamins" - Published New York 1922, First Edition

(a) Evidence from Observation upon Disease

"Observations (1) upon disease led to the conception of three or more substances of the vitamin type, two soluble in water and needed for the prevention of beri-beri and scurvy respectively, and one soluble in fats and so essential to a normal condition of healthy resistance that its absence from the diet results in a characteristic eye disease variously referred to as ophthalmia, xerophthalmia, conjunctivitis or keratomalacia.

While in this way the conception of the vitamins has arisen largely in connection and is intimately associated with that of the deficiency diseases, yet from the present standpoint an even greater interest attaches to them as a chemical substance occurring in natural food and having important functions in the normal processes of nutrition."

(b) Evidence from Experiments upon Normal Nutrition

"To Hopkins (2) is due the honor of having first made clear that natural food contains and normal nutrition requires some other substance or substances besides proteins, fats, carbohydrates and mineral matters. As early as 1906, he had determined and reported that "no animal can live upon a mixture of pure protein, fat and carbohydrate and even when the

(1) (2) Sherman H. C. and Smith S. L. -"The Vitamins"- Published New York,1922, First Edition

necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals and these contain countless substances other than the proteins, carbohydrates and fats." In his experiments, using rats as subjects, Hopkins found that the addition of small amounts of milk to diets otherwise composed of purified foodstuffs resulted in growth as Figs. 1 and 2 show, and that this was due to an alcohol soluble organic substance or substances in the milk and not any of its known constituents. Certain vegetables had the same property but in a lesser degree than milk."

"Prior (1) to 1911 none of the earlier workers attempted to name the essential substance or substances. In that year Schaumann (2) designated his active preparation "Activator," but the term did not come into widespread use. At the same time Funk (3) separated a potent product from rice polishings, and postulated the existence in foodstuffs of a number of such indispensable substances which he called "vitamins"."

(1) Kruse H.D., McCollum E.V. "Biochemical Investigations of Vitamin B." Physiological Reviews-Vol. 8, Pub. Baltimore, Md. 1928, p.-126-239
(2) Schaumann H. "Further Contributions to the Etiology of Beri-beri." Trans. Soc. Trop. Med. Hyg., Vol. 5, p. 59, Pub. London, England 1911
(3) Funk C. "On the Chemical Nature of the Substance which Cures Polyneuritis in Birds Induced by a Diet on Polished Rice." J. Physiol., Vol. 43, p. 395. Pub. 1911-1912, Cambridge University Press, England

"Hopkins (1) in 1912 called attention to the necessity of "accessory factors" in the diet for growth."

"Osborne and Mendel (2) demonstrated that similar growth-promoting property was possessed by their "protein-free milk," a powder prepared by removing the fat, casein and albumin from cow's milk and evaporating the filtrate to dryness. A little later it was found both by McCollum and Davis (3) and by Osborne and Mendel (4) that the fat of milk also possesses a growth-promoting substance which is not an attribute to the triglycerides themselves but rather of a fat-soluble substance. Both, the water soluble and the fat soluble growth-promoting substances are fairly soluble in alcohol which accounts for the fact that Hopkin's alcoholic extract of dry milk contained both of these essentials and supplied all that was needed for the growth of rats when added to his mixture of previously recognized foodstuffs."

(1) Hopkins F.G. "Feeding Experiments Illustrating the Importance of Accessory Food Factors in Normal Dietaries," J. Physiol., Vol. 44, p. 425, Pub. Cambridge University Press, England, 1912
(2) (3) (4) Sherman H. C. and Smith S. L. "The Vitamins" Pub. N.Y. U.S.A., 1922

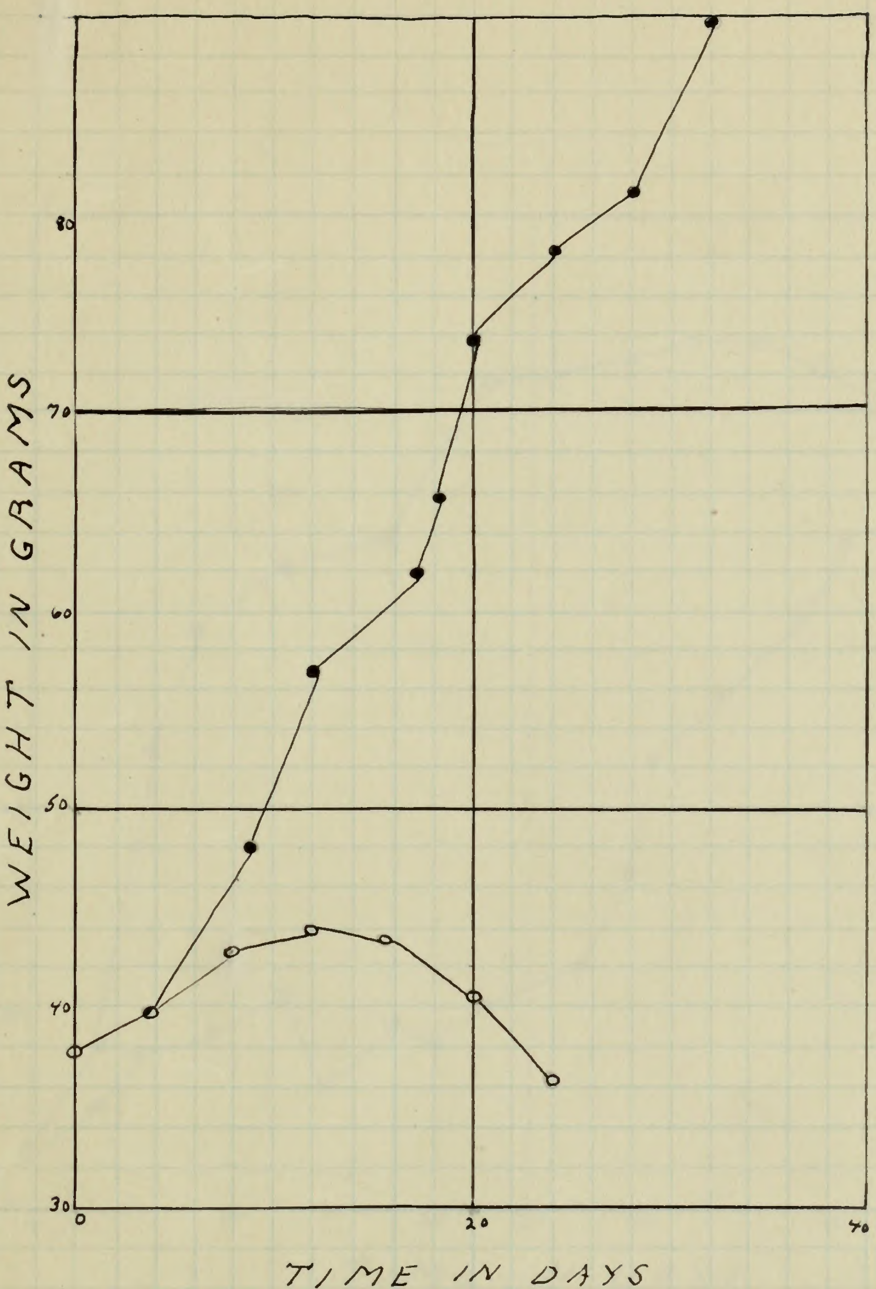

Fig I[(2)]

(1) Fig. 1[(2)] Page 12,"The Vitamins"- By Sherman H. C. & Smith S. L.
Published N. Y. 1922,First Edition

(2) Courtesy of Professor Hopkins and the Medical Research
Committee of Great Britain

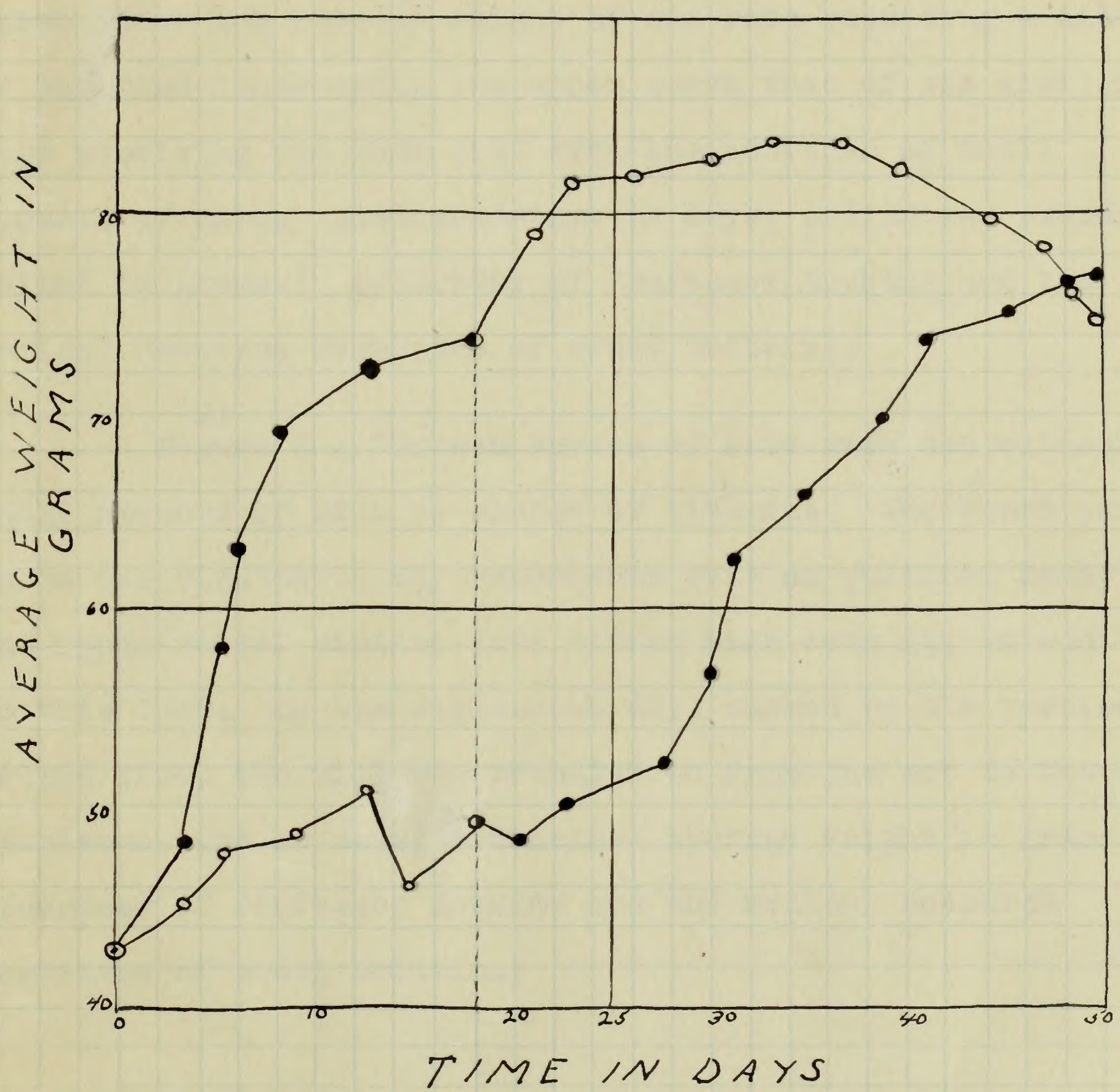

Fig II (1)

(2)
(1) **Fig. 2** **Page** 13,"The Vitamins"- By Sherman H. C. & Smith S. L. Published N. Y. 1922,First Edition

(2) Courtesy of Professor Hopkins and the Medical Research Committee of Great Britain

Explanation of:

(1) Figure 1 : "Growth curves of rats with and without the vitamins furnished by small amounts of milk. The lower curve shows the average weight of six rats receiving a diet of purified foodstuffs; the upper curve that of six similar rats receiving the same diet with the addition of small amounts of milk. Abscissae-time in days; ordinates- average weight in grams." (Courtesy of Professor Hopkins and the Medical Research Committee of Great Britain).

(2) Figure 2 : "Growth curves of rats with and without small amounts of milk as source of vitamins. The lower curve up to the eighteenth day represents rats on purified food; the upper curve, similar rats having milk each day in addition to this food. On the eighteenth day, marked by the vertical dotted line, the milk was transferred from one set to the other. Abscissae-time in days; ordinates- average weight in grams." (Courtesy of Professor Hopkins and the Medical Research Committee of Great Britain.)

(1) and (2) Page 12, "The Vitamins" -By Sherman H. C. and Smith S. L. Published N.Y.,1922, First Edition

"The theory soon gained currency and is still generally held that the water-soluble and fat soluble substances essential to growth are identical with the water-soluble and fat-soluble "vitamins" which prevent beri-beri and ophthalmia respectively. The addition of these two vitamins to a diet otherwise consisting of properly selected isolated foodstuffs appear to provide all that is needed for the growth of rats. The normal growth of babies and of young guinea-pigs requires, however, the feeding of sufficient amounts of antiscorbutic vitamins as well.

Thus normal nutrition, at least in the human and some other species, demands adequate supplies of all three of what are believed to be the same vitamins which are essential to the prevention of the three "deficiency diseases" of scurvy, beri-beri and ophthalmia."

(c) Terminology or Nomenclature

"There has been much controversy concerning the terminology or nomenclature of the first three vitamins.

Previous (1) to 1920, the names which seemed best to express the distinctive properties of the three vitamins were known as "antineuritic", "antiscorbutic", "antiophthalmic" (or "anticonjunctivitic") vitamin respectively. It seems, from a standpoint of chemistry and normal nutrition, a somewhat round-about procedure that a substance having an important role

(1) Sherman H. C. and Smith S. L. "Vitamins" - published N.Y., 1922, First Edition

in normal processes should be named according to the abnormal condition which arises when it is lacking. Moreover, the term "vitamine" has been criticized, both because it implies that these substances are amines which is not proven in any case and certainly not probable in all, and because the choice of "vita" as a specific term is thought by some to carry an exaggerated implication of unique responsibility for life and vitality; whereas other substances such as tryptophane are no less essential. However, the designation "accessory" suggested by Hopkins (1) is certainly too modest, since an accessory substance would be judged to be dispensable, whereas the indispensability of these substances is one of their most marked characteristics."

"To avoid these difficulties, McCollum (2) suggested that until such time as chemical names can be exactly assigned to them, these substances be known by alphabetical designations, qualified only by such statement of their solubilities as may seem helpful. In his earlier rations of supposedly purified foodstuffs, McCollum furnished water-soluble vitamin in the lactose fed. Hence, he at first concluded that the fat-soluble substance of butter, egg-fat, etc., was the only unidentified substance essential to normal nutrition. Thus, this became, in his alphabetical terminology, "fat-soluble A", and the water-soluble, although really discovered much earlier was designated as "water-soluble B"."

(1) (2) Sherman H. C. and Smith S. L. "The Vitamins", Pub. N.Y. U.S.A., 1922

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(1) (2) Sherman H. C. and Smith S. L. "The Vitamins", Pub. N.Y. U.S.A., 1922

"For a time McCollum consistantly opposed the view that a third "antiscorbutic" substance was also necessary, but as the evidence of its existence became known, this latter was included in the alphabetical terminology as "water-soluble C". The unfortunate features of this system of terminology are the lack of harmony between historical and alphabetical sequence and the somewhat exaggerated emphasis upon apparent solubility."

"In 1920, Drummond (1) suggested that the alphabetical designations now familiar be retained, but without the antecedent statement of solubility, and the original designation of Funk (2) be retained, but the final "e" be dropped, so that the resulting word vitamin shall carry no implication as to the chemical constitution of the substance. This suggestion was adopted and the first three substances to be discovered are designated as vitamin A, B, C, respectively; and any others whose existence may be demonstrated before they are chemically identified, can be labelled alphabetically in chronological order."

Thus, a while later the antirachitic vitamin was discovered and classified according to the above category and designated as vitamin D. A while after the discovery of vitamin D, another substance, vitamin E, was discovered and found to be necessary in the reproductive system of animals. To date, vitamin F and vitamin G have been discovered. Vitamin F is

(1) (2) (3) - Sherman H. C. and Smith S. L. - "Vitamins" - Published N.Y., 1922, First Edition

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(1) (2) (3) - Sherman H. C. and Smith S. L. - "Vitamins" - Published N.Y., 1922, 2nd Edition

associated with the antineuritic property. Vitamin G has been designated by some writers as the antipellagric vitamin. The Medical Research Committee (1) has decided to designate vitamin B or B1 which is vitamin F as the antiberi-beri vitamin and PP or B2, which is vitamin G, as the pellagra preventing vitamin.

"Although (2) the vitamin theory of the present day had its origin in the study of the antineuritic substance and the fundamental conception of the vitamins has so largely grown up around the properties of the substance to which the name was first given, the discussion of the antineuritic vitamin shall take place secondly, and the differentiation of the other vitamins shall follow alphabetically and so in order of discovery (with the exception of vitamin A preceding B)."
(C 1)

Terminology of Vitamin A (3)

"This is formed in the actively growing portions of plants and is then taken up by grazing animals. This vitamin is known as the fat-soluble vitamin, since it is found in the animal body in association with fat. It is a complex compound of carbon, hydrogen, and oxygen, but has no nitrogen. Vitamin A is found in high concentrations in egg-yolk, milk fat, and cod-liver oil. If animals are derived of vitamin A, their nutritive condition suffers so that they go into a state of decline in

(1) Plimmer, R.H.A., and V.G.-"Food, Health, Vitamins"-
Published London, 1928-Third Edition

(2) Martin, E.G. "Experimental Physiology" -"The Human Body,"
(3) Published N.Y., 1926 - Eleventh Edition

which they show marked susceptibility to certain kinds of diseases (an eye disease almost invariably develops in laboratory animals on an A-free diet). Young animals show a marked retardation of growth when fed a diet deficient in A, and death results from prolonged complete deprivation. This retardation of growth may simply be an indirect effect of the general malnutrition bringing on A- deficiency; yet its effect is so marked as to earn for this vitamin the name of the growth-promoting vitamin.

Growing children should be fed eggs, whole milk or cream, or butter as oleomargarine which must be made from fish oils or animal oils, and not made from vegetable oils- to prevent its disease effects. The occasional administration of cod-liver oil is an aid to insure good health of the child, yet this is given more for its content of another vitamin- vitamin D."

"According to Zilva and Miura (1), cod-liver oil contains 250 times as much vitamin A as butter, and 3 milligrams of this oil per day is sufficient for rats. Drummond, Coward and Watson (2) found that certain samples of butter contained no more vitamin A than refined vegetable oils

(1) Zilva S.S., Miura M. "A Note on the Activity of Fat-Soluble Accessory Factors in Cod-Liver Oil and Butter," The Lancet, Vol. I, p. 323, Pub. 1922, London, England.

(2) Drummond J. C., Coward K. H. and Watson A. F. "Notes on the Factors Influencing the Value of Milk and Butter Sources of Vitamin A," A. Biochem. J., Vol. 15, p. 40, Pub. Waverly Press, Baltimore, Md.

The above data undoubtedly explains the difference observed in the value between cod-liver oil and butter in the treatment of rickets (1)."

(C2)
Terminology of Vitamin B

"Vitamin B (2) was the first of the vitamins to be discovered and it was found that lack of vitamin B produced a deficiency disease called neuritis. The deficiency disease, beri-beri became prevalent among rice eating people when they began to substitute, chiefly, rice hulled clean by power machinery for the same food hulled in cruder fashion but less so.

By adding water extracts of rice hulls to the diet in small quantities, it was found that the disease beri-beri could be prevented or cured. Vitamin B is a nitrogen-containing compound belonging to the chemical class of "amines"."

"Vitamin B is soluble in water and hot ethyl alcohol (3). The great water-solubility of this vitamin suggests that in the cooking of fresh foods in water a considerable amount of this substance may pass into the water, and that the latter should therefore be consumed with the cooked food wherever possible."

"The antineuritic vitamin occurs abundantly in yeast, in the hulls of grain, eggs, milk, and in the green parts of plants. The bodies of animals contain little of it; therefore carnivirous animals do not obtain much of it, except through the nibbling of grasses as dogs and cats do. Since eggs and

(1) Funk C. "The Vitamines" Translation, H. Dubin, Pub. Waverly Press, 1922, Baltimore, Md., Second Edition
(2) Martin, E.G. -"Experimental Physiology" - The Human Body," Pub. 1926, N.Y. U.S.A. - Eleventh Edition
(3) Voegtlin C. "The Metabolism of Vitamins" "Endocrinology and Metabolism," Vol. 3, Pub. 1922, London, Eng.

milk contain rich supplies of B, the vitamin must pass into these from the maternal bodies.

Humans existing on ordinary mixed diet rarely suffer deficiency of B as to develop beri-beri; they may, however, show other symptoms indicative of its insufficiency. Oftentimes, the undernutrition attributed to B is purely due to the lack of appetite it brings about and only indirectly to the vitamin itself. Fresh milk, green foodstuffs, as lettuce, and celery, afford the body the needed amounts of vitamin B; if these are not sufficient, a cake of yeast a day will do."

(C3)
Terminology of Vitamin C

"This is the (scurvy-preventing) antiscorbutic vitamin (1). People depending on salt meat and hard tack have suffered from scurvy, mostly those on long voyages or expeditions. For a long time, it has been know that scurvy can be prevented by eating fresh fruit and most fresh vegetables. Vitamin C is soluble in water and alcohol (2); it is particularly abundant in juices of oranges, lemons and tomatoes, also in the growing shoots and immature vegetables. If the food of cows consists largely of fresh grass, their milk will contain C in plenty, but if it consists of hay or grain, it contains little or none. This is important because infants suffer frequently from a form of scurvy when not given sufficient C. Mothers' milk may or may not contain C according to whether her diet contains fruit

(1) Martin, E. G. - "Experimental Physiology - The Human Body," Pub. N.Y. 1926 - Eleventh Edition

(2) Harden, A., Zilva S.S. "Susceptibility of the Anti-Scorbutic Principle to Alkalinity," The Lancet, Vol. 2, p. 320, Published 1918, London, Eng.

or not. So the practice of giving infants orange juice owes its value to the presence of Vitamin C, yet the practice arose long before the facts about the vitamin became known."

(C4)
Terminology of Vitamin D

"The antirachitic vitamin may be generated in almost any animal or vegetable fat by exposure to ultra-violet radiation, it may or may not be of plant origin (1). All other vitamins are exclusively of plant origin. Vitamin D is fat-soluble. "Rickets" (rachitis) is a disease prevalent among children suffering from a form of malnutrition in which sufficient lime is not deposited in the growing bones to give them the needed strength and rigidity. The disease may develop even when the diet contains sufficient lime salts, and also all the phosphoric acid needed for the formation of the insoluble calcium phosphate."

"Vitamin D apparently controls the deposition of the lime salts and phosphates in the bones and teeth and compensates for any irregularity in the proportion of lime salts and phosphates (2). It has been known that cod-liver oil contains vitamin D. Other fats which contain vitamin D. and prevent rickets are Beef Suet, Butter, and Egg Yolk."

"The observation was made that children who play in the direct sunlight without glass intervening, do not develop rickets, or get well of it, if they have it without the usage of cod-liver oil. The answer to these two agencies is that

(1) Martin E.G.- "Experimental Physiology" - "The Human Body," Pub. 1926, N.Y., Eleventh Edition
(2) Plimmer R.H.A., Plimmer V.G. "Food, Health, Vitamins", Pub. 1928, London, Eng.

vitamin D is formed in most any kind of fat if subject to the ultra-violet rays. The invisible rays of the sun are abundant in the air, however, glass being opaque to the rays they are cut off (1). If the skin is directly exposed to the sun's rays, enough vitamin will be formed, so that the bones will deposit their lime normally. This last phase is dependent upon a sufficient amount of the ordinary fat foods circulating in the blood vessels of the skin. Cod-liver oil contains the vitamin,D, since fish are exposed to the rays of the sun or eat food that has been exposed to it."

(C5)
Terminology of Vitamin E

"This vitamin has recently been discovered to be necessary in the reproductive process of animals (2). The experiment was carried out on white rats; it was found that the germinal tissues suffer impairment if E is lacking in amount; another test showed that the embryos may appear to start a normal development within the uterus of the mother, but when vitamin E is deficient the embryos die in a short time and are reabsorbed into the blood of the mother. As yet,the direct explanation of this problem has yet to be found. Vitamin E is found in the germ of wheat and some other grains, but, in no other part of the grain. It is found in lettuce and alfalfa, in liver, and to a certain extent in milk-fat."

(1) (2) Martin E.G. - "Experimental Physiology" - "The Human Body," Pub. 1926, N.Y. Eleventh Edition

"So far as the nutrition of man and animals is concerned, the ordinary mixed diet containing meat, eggs, green leaves and seeds, supplies plenty of vitamin E (1)."

(C6)
Terminology of Vitamin F and Vitamin G or B1 or B2 or PP

"These two vitamins have recently been discovered as being closely linked with vitamin B whose many effects produced have led to repeated suggestions that more than one dietary essential may be involved, until at length it seems to be conclusively demonstrated that what was once formerly designated as vitamin B does compose at least two properties of food that are equally independent, and essential to well being (2).

The designation of the factors is still a matter of considerable debate among biochemists. In Great Britain the current usage refers to vitamins B1 or B2; in this country there is a growing tendency to assign new letters, F and G, or PP to the individual factors so as to avoid the implication that they are fragments of a simple compound. One of them (B1 or F) unquestionably represents the long recognized antineuritic property; the other (B2 or G or PP) is equally essential and has been recognized by some writers as the antipellagric vitamin. The time has come when foods need to be re-examined with reference to their content of vitamin F and G."

(1) Plimmer R.H.A., Plimmer V.G. "Food, Health, Vitamins", Pub. 1928, 3rd Edition, London, England
(2) Editorial, Journal A.M.A., Volume 91, 1928, p. 1720 to 1722, Pub. Chicago, U.S.A.

"The objection to this nomenclature is that no special physiological function is assigned to Eijkman's classical antiberi-beri vitamin, which has for the last fifteen years been known as vitamin B, and the universal recognition of which has been largely responsible for the stimulation of vitamin research (1)."

"Yeast contains both vitamin F and G, in considerable abundance; indeed, it has been possible to make separations of yeast fractions neither of which alone will promote well being and growth without the other, on an otherwise adequate diet (2). (A discussion on yeast separation will be given shortly).

Wheat and its products are comparatively poor in vitamin G factor, hence it may properly be supplemented with supplies of the lacking factor. Corn likewise appears to be poor in the antipellagric potency.

According to recent studies, milk shows reverse relations. Thus, Hunt and Kraus have found at the Ohio Agricultural Experiment Station in Worcester that milk from cows under winter feeding conditions is potent in the antipellagric vitamin, vitamin G, and relatively poor in the antineuritic vitamin, vitamin F (3).

Again, science demonstrated that the conventional combination of cereal and milk has a nutritional justification as well as a gustatory one. Each food supplies something essential that the other lacks in adequate relative abundance."

(1) Sure B. "A Differentiation of the Vitamin B Complex in Rice Polishings as Evidenced in Studies of Lactation" - J. Biol. Chem., Vol. 80, p. 297, Pub. 1928, Baltimore, Md. U.S.A.
(2) (3) Editorial, Journal A.M.A. Vol. 91, p. 1720-1722 Pub. 1928, Chicago, Ill. U.S.A.

Chapter II

Vitamins in Plants

"A substance that promotes growth in yeast as vitamins do in animals, has been found; this substance is called "bios", deomomstrated to be really two substances and one of these "bioses" has been prepared in pure form in the laboratory of Professor W. Lash Miller, of the University of Toronto. It proves to be a form of a seldom studied but long known chemical compound, inosite. "Bios" is then a vitamin necessary for the growth of yeast, but it is not necessary for growth in animals. It may then be considered as analogous in its importance to that of the vitamins which are required for animal growth (1)."

(a)
Separation of the vitamin-like "bios"

"In 1901, long before vitamins were discovered, a Belgian chemist named Wildiers found that yeast needed for growth small quantities of a special substance of unknown chemical composition (2). Wildiers could not isolate this substance. He gave to the unknown term the word "bios", which is the word for life. After the separation of vitamins, scientists began to take interest in this vitamin-like material needed by yeast, but it yet defied separation and chemical analysis.

"Then various researchers in the laboratory of Professor Miller began a systematic attach on the problem. One of them discovered that if a "bios" solution were shaken up with fine char-

(1) Editorial, Science n s 68: Sup. 10, July 6, 1928
(2) Tanner F.W. Devereux E.D. Higgins F.M. "The Multiplication of Yeasts and Yeast-Like Fungi in Synthetic Nutrient Solutions," J. Bact., Vol. 11, p. 45, Pub. 1928, Baltimore, Md.

coals some of the "bios" vanished into the charcoal and what was left could not help yeast to grow (1).

That part that was left could also be cleared out of the solution by other chemical means. This indicated that there was not one "bios" but two, thus the names "bios I and "bios" II came into use. The latest advance has been to crystalize "bios" I, crystals are the chemist's test for purity; a mixture will not crystalize. The crystals obtained have been analyzed and shown to contain the same proportions of carbon, hydrogen and oxygen as common glucose, but the chemical arrangement is much more complex. "Bios" I, or inosite as it has now been proved to be, is abundant in young vigorously growing plant shoots. The German investigators who first studied inosite obtained their material from bean sprouts. The Canadian scientists who purified "Bios" I and established its chemical identity bought up large quantities of tea siftings for their raw material."

"Ralph W. Kerr refers to "Bios" I as alpha bios and "Bios" II as beta bios (2). "Bios" II is found in yeast, alfalfa, and tea siftings."

(b)
Greeness of Vitamin A in Plant Tissue

"We can now consider greeness and vitamin A in plant tissue. Experiments conducted in England have demonstrated that the inner whitish leaves of the cabbage head are very poor in

(1) Editorial, Science n s 68: Sup. 10, July 6, 1928
(2) Kerr R. W. "The Isolation of Beta Bios "-Proc. Soc. for Exper. Biol.,Vol. 25, p. 340, Pub. Utica, N.Y., 1928

vitamin A, while the outer green leaves are richer in this respect. Also, it has been shown that the stalks of bleached celery contain no more than traces of vitamin A (1). This vitamin is also deficient in the inner leaves (the bulk of the head) of head lettuce, and in the innermost yellowish leaves is probably entirely lacking. The vitamin A value of green and bleached asparagus in the diet of white rats has been determined at Michigan State College; the results of the experiment have not, as yet, been published in technical form, but were reported at the meeting of the American Association for the advancement of science held at Nashville, Tennessee, in December 1927. These experiments demonstrated that when growing asparagus is covered over in the row with soil and the tips cut before they reach the surface and become green by exposure to the sunlight, their value in terms of vitamin A is low. Animals fed on the fresh white tips lost weight, became badly diseased and died before the end of the sixth week, while other animals receiving an equal amount of fresh green tips of vegetables were healthy, vigorous and gained weight at the rate of about five grams per week."

(1) Crist J. W., Dye M. - "Greeness and Vitamin A in Plant Tissue," (Scientific Monthly) Pub. N.Y., U.S.A.,August 28, 1928

Chapter III

Vitamins in Animals

While new facts about the vitamins necessary for the health and happiness of the human race come to light nearly every day, entomologists have been endeavoring to find which, if any, vitamins are needed to keep up health of the insect world.

(a)

Insects

"In whole wheat flour we find vitamin A and B, and it was this flour that was used in the experiment along with the Mediterranean flour moth that thrives as a pest on the flour (1). The experimentation was carried on by Dr. Charles H. Richardson of the United States Bureau of Entomology. In whole wheat flour, the larvae of the moth lived and flourished happily but in the same kind of flour from which a substance, believed to be vitamin A, was extracted by chloroform, relatively few larvae developed into full-grown moths. In highly milled flour, from which most of the growth-producing vitamin B is removed by the processes of manufacture, the growth rate was also poor. With the addition of small quantities of yeast, a rich source of vitamin B, the number of larvae that reached maturity increased. Thus, the reactions of the flour moth toward vitamins A and B checks very well with the requirements of laboratory animals and human beings. Further

(1) Editorial, "Vitamin Requirements of Insects" - Science n s 68: Sup. 12, Published N.Y., U.S.A., July 27, 1928

studies on this problem with other insects will be of great interest from the point of view of comparison of insect physiology with that of higher animals. Practically, it will effect the control of the insect pests, since any factor that might render poisoned bait for harmful insects more attractive would assume great economic importance."

(b)

Rats and Vitamin E

"Rats can be normally reared on a normal rate of synthetic diet which contains pure casein (milk protein), starch, lard, milk, mineral salts and yeast, that is on a diet containing all the known essentials for nutrition, but on this diet there is little or no reproduction. Rats do not need vitamin C (1).

The sterility of the animals is not overcome by increasing the proportion of protein, of yeast, nor by the addition of cod-liver oil - a different salt mixture has no better effect.

The introduction of lettuce or of dried leaves, or of whole wheat or oats, or egg yolk, or liver into the diet gave the stimulus for reproduction. This fact was first announced by Evans and Bishop in 1922 and has been confirmed by other workers in America (2). Since all known factors were given in the synthetic diet which sufficed for growth, the conclusion was that another vitamin was necessary for reproduction. This is called vitamin E.

Experiments with rats have been repeatedly made to secure reproduction by altering proportions without any real

(1) (2) Plimmer, R.H.A. and V.G. - "Food, Health, Vitamins," Published London, England, 1928, Third Edition

success in the production of young. On these diets reproduction has been secured on adding wheat germ oil, or hardened cotton seed oil. Neither of these oils contains vitamin A and D which are present in cod-liver oil. A third vitamin is,thus, present in some fats of different origin.

The examination of the fats for vitamin E has shown it to be present in the unsaponifiable portion. Further examination of this material has shown that it is not in the part in which one finds vitamin D, but in that portion in which one would find vitamin A.

The two are not identical because vitamin A is easily destroyed whilst E is not so quickly destroyed. This fraction, not containing vitamin A, if added to the diet, allows of reproduction in rats on the synthetic diet.

The ordinary mixed diet of man and animals supplies plenty of vitamin E. The addition of vitamin E to foods has been shown not to lead to any extra reproduction of young. A well-balanced diet of all the essentials leads to good reproduction and upbringing of young.

It has also been observed that mother rats, although bearing young, are not always able to rear them from want of sufficient milk. Another vitamin, F, was at one time supposed to be concerned in lactation. It has, however, been found that extra vitamin B in the diet improved lactation. In fact three to five times as much vitamin B is now wanted by the mother. There is, thus, no need at present for a special vitamin for lactation and rearing."

Chapter IV

Quantitative Differentiation of Vitamins A and D

"This method of differentiation of vitamins A and D is based upon the earlier work of Drummond, Coward (1), and their associates, and now involves the use of experimental animals (rats) of accurately known age and nutritional history (2). The rats are placed when four weeks old upon a basal diet excellently adapted to the needs of the animals in all other respects but devoid of vitamin A, upon which diet they are kept until growth has ceased because of depletion of their bodily stores of vitamin A; then leaving some on the basal diet only as negative controls, others are fed different fixed allowances of the food to be tested as their only source of vitamin A until it is determined what daily allowances of their food under investigation is required to support an average rate of gain of 3 gm. per week during the experimental period in a standard test animal prepared as indicated; therefore this daily allowance of food is said to have furnished 1 unit of vitamin A. This method with a few modifications has been incorporated in the later revision of the United States Pharmacopoeia as an official procedure for the testing of cod-liver oil for its vitamin A value. In order that such determination of vitamin A has been quantitatively uninfluenced by vitamin D (or antirachitic

(1) Drummond, J.C., Coward, K.H., and Handy, J., Jour. of Biochem., "On the Technique of Testing for the Presence of Vitamin A," Vol. 19, p. 1068, Pub. 1925, Cambridge University Press, London, Eng.

(2) Sherman, H.C., Hessler, M.C. - "Experiments on the Quantitative Differentiation of Vitamin A and D," Jour. of Biol. Chem., Vol. 73, Pages 112-120, Pub. 1927, Balt., Md., U.S.A.

value of the material which is being tested for vitamin A), it is essential that the vitamin D requirement of the test animals shall be provided for, at least to the extent needed for the maintenance of a rate of growth of 3 gm. per week. According to present knowledge, this antirachitic or vitamin D requirement may be met in any of four ways; by irradiation of the animal, by irradiation of its food, by the addition of irradiated cholesterol to the food, or by so feeding the families from which the test animals are drawn that they will have acquired bodily stores of vitamin D sufficient to meet their needs for the time and conditions of the vitamin A experiment, when carried out as described.

The last named condition appears to have been fulfilled in most of the work of this laboratory upon the testing of foods for vitamin A even before Steenbock's discovery that the antirachitic factor is also an essential for growth."

(a) Experimental Method (1)

"The experimental animals were protected from such shortage of the antirachitic factor as seems to have inhibited growth in some of the cases described by Steenbock and Nelson (2) in two ways. Firstly, pursuing the experimental investigations of the English, the growth of the animal was restricted during the experimental period, in most cases to a gain of approximately 3 gms. per week. The fact that very little growth is permitted by the vitamin A intake naturally has the effect of conserving such shortage of vitamin D as is contained within the body of the ex-

(1) Sherman H.C. and Hessler M.C. - "Experiments on the Quantitative Differentiation of Vitamin A and D," Jour. of Biol. Chem., Vol. 73, p. 112-120, Pub. 1927, Balt., Md., U.S.A.

(2) Steenbock H. and Nelson M. - "Light and its Relation to Ophthalmia and Growth", J. Biol. Chem., Vol. 56, p. 335, Pub. 1923, Waverly Press, Balt., Md., U.S.A.

perimental animal. Secondly, this bodily store of vitamin D is no doubt made relatively larger in these animals than in those of Steenbock by the difference in the diets of the families from which the young rats are drawn. In the diet used by Steenbock, the vitamin A requirement is supplied chiefly by dried alfalfa leaves which, as his work shows, are poor in vitamin D; whereas in the diet chiefly used in this experiment, whole milk is the main source of vitamin A, and this, at the time of experimentation, supplies more vitamin D than does the dried alfalfa; so the animals raised on the diet in which vitamin A is supplied by whole milk undoubtedly acquired much larger bodily stores of vitamin D than those reared on the diet whose chief source of vitamin A is dried alfalfa.

Clinical reports of rickets occurring in milk-fed infants have apparently led to a broad failure of appreciation of the antirachitic value of the fat or milk and butter. McCollum and his coworkers (1) have included a small percentage of butter in their rickets producing diet, but have recently pointed out that this limited amount of butter fat sometimes gives enough vitamin D to prevent the development of rickets; and Mellanby (2) emphasizes the markedly favorable influence of butter fat upon the development and calcification of the bones of the puppies which he used as experimental animals in his studies of the rachitic and antirachitic effects of foods."

(1) McCollum E.V.; Simmonds N.; Becker J.E.; Shipley P.G.-"Studies on Experimental Rickets," Jour. of Biol. Chem., Pub. 1926, Balt., Md., Vol. LXX, p. 437

(2) Mellanby E., British Medical Research Council, Special Rep. Series, No. 93., "Experimental Rickets", Pub. 1925, 24, London, Eng.

"In the work of Dr. Munsell (1) in the laboratory of Sherman and Hessler, it had been noted that among animals which had been equally retarded in growth by shortage of vitamin A, the bone development appeared somewhat better in those whose limited allowance of vitamin A had been furnished in the form of butter or whole milk than in those which had received the same limited amount of vitamin A in the form of certain other foods, notably carrots. From this conclusion, it would appear that the most rigorous test of the question as to whether the method of Sherman and Munsell (2) effects a quantitative differentiation between vitamins A and D would be afforded by feeding carrots as sole source of vitamin A (and sole immediated food source of vitamin D) and determining whether under these conditions the irradiation of a part of the experimental animals would increase their rate of gain in body weight; furthermore, an advanced step was made toward the quantitative work with the antirachitic factor, in comparing the gains in body calcium. As a further check upon results and conclusions, there was also included in their work some additional experiments upon gain in weight with and without irradiation when the limited allowance of vitamin A was supplied by butter fat.

In a comparison of 13 animals,so receiving their vitamin A in the form of butter fat,with 14 parallel cases fed in the same way,but receiving ultra-violet irradiation in addition, the average gain in weight for the 8 weeks experimental period was 6 gms. less

(1) (2) Sherman H.C. and Hessler M.C. - "Experiments on the Quantitative Differentiation of Vitamin A and D", Jour. of Biol. Chem.,Vol. 73, p. 112-120, Pub. Balt., Md., U.S.A., 1927

for the irradiated animals than the non-irradiated animals. This confirms the view that shortage of vitamin D does not influence the weight curve obtained in the testing of foods such as butter for vitamin A by the method of Sherman and Munsell. It also indicates, for the purpose of the present investigation, a difference of 6 gm. gain in body weight in the average of a group of a dozen or more of experimental animals should possibly be regarded as accidental."

(b) (1) DATA TABLE I

Average of Body Weight and Body Calcium in Test Animals Receiving Limited Allowances of Vitamin A Containing Food, without and with Ultra-Violet Irradiation

Sources of Vit. A & D	Gain in Body Wt.		Final Calcium Content of Body	
	No. of cases	Average gm. in 8 weeks	No. of cases	Average % of Ca.
Carrots, 0.3 gm. per week	27	19	24	1.22
Same plus irradiation	29	23	30	1.28
Carrots, 0.6 gm. per week	22	31	21	1.25
Same plus irradiation	22	39	18	1.22

"Table I summarizes the net effects upon body weight and body calcium, of the 8 weeks of experimental feeding without and with ultra-violet irradiation. Only the averages for the principal series are tabulated. If the comparison is confined to cases of animals of similar size, the effect of irradiation upon the average gain in weight becomes more pronounced, that is, 8 and 11 gm. instead of 4 and 8 gm. respectively. This is in all probability significant.

(1) Sherman H.C. and Hessler M.C.- "Quantitative Differentiation of Vitamin A and D", -Journal of Biol. Chem., Vol. 73, Pub. 1927, Balt., Md., U.S.A., From Table I. p. 116

In a comparison of the results of the 2 series in which butter and carrots respectively were fed, their outstanding significance would seem to be that (as suggested by Munsell) in the determination of vitamin A in butter fat (by the method used in the laboratory of Sherman), there is, plainly, no disturbing shortage of vitamin D, whereas in the determination of vitamin A in carrots by the same method, there appears a possibility that, in the absence of irradiation of the weight curve employed as a means of measuring the amount of vitamin A supplied by the food, may perhaps be influenced by a simultaneous shortage of vitamin D.

The x-rayed knee joints of animals, the line tests of the tibia of these animals, and histological examinations of their rib junctions showed, although the examinations were not made in sufficient large numbers of cases to justify any attempt at a quantitative (statistical) interpretation, mainly that there was no well-marked difference in any of these respects between the irradiated and the non-irradiated animals; but that all these examinations taken together - and perhaps most clearly the general trend of the line tests - may be taken as affording a slight indication of shortage of vitamin D in the cases in which carrots served as the sole food source of fat-soluble vitamin and no irradiation was supplied. Returning to the last two columns of the Table I to investigate whether this qualitative impression in bearing out the quantitative determinations of body calcium, it is found, that compared in groups of 18 to 30 animals each, the average percentage of body calcium seems in the first series to have been slightly increased by the irradiation, but, in the second series, there is as large an average difference in the opposite direction which can only be interpreted

In a comparison of the results of the 2 series in which butter and carrots respectively were fed, their outstanding significance would seem to be that (as suggested by Russell) in the determination of vitamin A in butter fat (by the method used in the laboratory of Sherman), there is, plainly, no disturbing shortage of vitamin D, whereas in the determination of vitamin A in carrots by the same method, there appears a possibility that, in the absence of irradiation of the weight curve employed as a means of computing the amount of vitamin A supplied by the food, may perhaps be influenced by a simultaneous shortage of vitamin D.

The x-rayed knee joints of animals, the tibia bones of the tibiae of these animals, and histological examinations of their rib junctions conducted, although the examinations were not made in sufficient large numbers of cases to justify any attempt at a quantitative (statistical) interpretation, result that there was no well-marked difference in any of these respects between the irradiated and the non-irradiated animals; but that all these examinations taken together - and perhaps most clearly the general trend of the line tests - may be taken as affording a slight indication of shortage of vitamin D in the cases in which carrots served as the sole food source of fat-soluble vitamin and no irradiation was supplied.

Returning to the last two columns of the Table I to investigate whether this qualitative impression in bearing out the quantitative determinations of body calcium, it is found, that compared in groups of 15 to 30 animals each, the average percentage of body calcium seems in the first series to have been slightly increased by the irradiation, but, in the second series, there is as large an average difference in the opposite direction which can only be interpreted

as due to chance or physiological variability of the animals.

The animals of all four of the groups here considered had been inhibited in growth by the shortage of vitamin A in their food, so any attempt to compare the data in the Table with strictly normal values for body calcium is complicated.

In all of these cases, as in similar cases previously studied, it was found that the amount of calcium in the body of an animal whose growth had been so retarded, was between the normal for the age and the normal for the weight."

(c)
Factor for Estimating the Total Calcium Content of the Body from the Calcium Content of the 2 Femurs

"If ribs, tibia, or other parts of the animal are used for other types of test the direct determination of the total calcium of the body is not possible; however, data of both types for the same animal are desirable (1). It was, therefore, decided in connection with this investigation to establish a factor by means of which, from a determination of the amount of calcium found in the 2 femurs, the calcium content of the entire body can be calculated. 56 determinations of femur calcium and of the total body calcium of the same animals were used for the establishment of this factor. The mean value found was 14.138 with a probable error of ± 0.135 a standard deviation of 1.500, and a coefficient of variation of 10.6 percent. The limits of value for this factor which would cover a range of four times the probable error of the means are, therefore, 13.60 to 14.65.

(1) Sherman H.C. and Hessler M.C. - "Quantitative Differentiation of Vitamin A and D," - Jour. of Biol. Chem., Vol. 73, Pub. 1927, Balt., Md., U.S.A.

In investigations similar to these, a convenient approximation of the total calcium content of the body may be secured by multiplying the femur calcium by 14.4. The 56 cases, from which this average is secured, were all young rats 80 to 120 days old, whose growth had been inhibited in varying degrees by shortage of vitamin A. The ratio of femur calcium to total body calcium in rats of different ages and nutritional histories is also being investigated in the laboratory where H. C. Sherman and M. C. Hessler performed this work."

(d) Summary of the Experiment

"In experiments carried out from the laboratory for the quantitative determination of vitamin A in foods, it has been found that irradiation of the test animals with the mercury-vapor quartz lamp does not produce any large increases in the growth of animals receiving limited amounts of vitamin A (1). This may be explained on the ground that the animals contain on the average a nearly sufficient bodily store of antirachitic vitamin to carry them through the test period where growth is limited as in the method employed. This is confirmed by a study of the bone structure, which showed few and minor deviations from the normal x-ray picture, line test, and histological appearance.

Under conditions of their experiment, the weight curve employed as a measure of the vitamin A content of the food under investigation was not influenced by shortage of vitamin D when the food was butter fat; but was somewhat influenced when the food was carrot. Evidently the butter fat contained more vitamin D in

(1) Sherman H.C. and Hessler M.C. -"Quantitative Differentiation of Vitamins A and D," - Jour. Biol. Chem., Vol. 73, Pub. 1927, Balt., Md., U.S.A.

the test animals have been established by previous experimentation, irradiation or the feeding of irradiated food to insure an adequate supply of vitamin D is a wise precaution. The extent of the differences which this may make varies with the stock diets used in different laboratories.

The animals which had received their limited allowance of vitamin A in a form which, as the result of previous work, was judged most likely to involve a shortage of vitamin D, were investigated further by quantitative determinations of body calcium. The results do not justify a positive conclusion as to whether or not the irradiation influenced the percentage of calcium in these cases. In one series of experiments, irradiation appeared to result in a slight increase in the percentage of body calcium; however, in a second series this result was not confirmed.

It is possible that the degree of calcification in animals which had been for 12 weeks on a diet that markedly retards growth is less delicate than would be a test for calcification at an earlier stage, and perhaps it may be a less quantitative indication of a shortage of vitamin D than the weight curve under adequately controlled conditions. Further studies of this question are under way.

With rats, as experimental animals, the factor by which femur calcium could be multiplied to obtain total body calcium was in a mean of 56 cases, 14.4."

Chapter V

Quantitative Determination of Vitamin B

The following study presents briefly the results obtained by the comparisons of quantitatively conducted feeding experiments and expressing the value of each material as tested in terms of the response obtained in a standard test animal (1).

(a) Experimental Procedure

The experimental procedure is as follows (1): "healthy and normally growing white rats of 28 to 29 days of age, from families of known breeding and nutritional history are placed in individual all metal cages with raised wire screen floors to prevent access to excreta, and are fed during an experimental period of 8 weeks with predetermined graduated allowances of the material to be tested as source of vitamin B, while furnished (ad libitum) with a basal diet adequate for all nutritional needs of the rat but devoid of vitamin B. The material under test, thus, becomes the sole source of the one vitamin for which it is being tested, and the concentration of this vitamin in the material may be judged to be inversely proportional to the amount of material which need be fed, if the test animal and all the conditions of the test are quantitatively standardized and controlled. Each animal is weighed at least once each week, and the amount of food consumed during each weekly period is recorded."

(1) MacArthur, E.H. and Sherman, H.C. - "A Quantitative Study of the Determination of Vitamin B," - Jour. of Biol. Chem., Vol. 74, p. 107-115. Pub. 1927, Balt., Md., U.S.A.

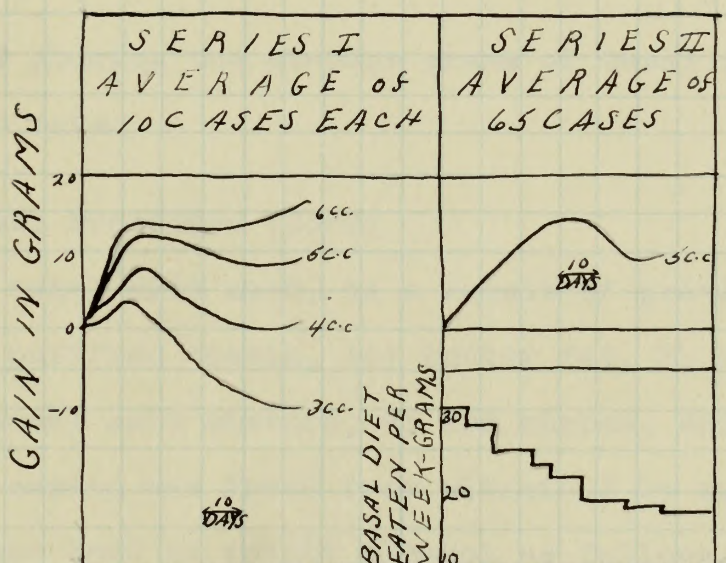

Fig III[1] Series I and Series II

(1) From Figure 1, Series I and Series II, Page 109, MacArthur, E.H. and Sherman, H.C. - "A Quantitative Study of the Determination of Vitamin B"- Jour. of Biol.Chem., Vol.74, Pub. 1927, Balt., Md., U.S.A.

Explanation of:

Figure III, Series I, curves showing the average gains in weight, of ten directly comparable cases each, made by rats fed varying dosages of evaporated milk as sole source of vitamin B.

Series II. Above a composite curve of 65 cases, showing average gains made by rats receiving the dosage of vitamin B which is probably best adapted to its quantitative measurement.

Below, a chart showing the average grams of basal diet eaten per week by the 65 rats.

(a) Experimental Procedure (Cont)

The basal diet used, as a result of previous investigation, consisted of: purified casein, 18; butter fat, 8; cod-liver oil, 2; Osborne and Mendel salt mixture, 4; and starch, 68% (1)."

The casein was freed from vitamin B by extraction in the cold with 60 per cent by weight alcohol as follows: "Each 200 gm. of casein was mixed for ½ hour with 1 liter of the alcohol by means of a mechanical stirrer and then allowed to stand for 5½ hours, filtered on a buchner funnel, and washed with 500 c.c. of 60% alcohol. The casein was again treated with 1 liter of 60% alcohol and allowed to stand for 18 hours, then filtered and

(1) Sherman H.C. and Spolm A.J. - "A Critical Investigation and an Application of the Rat-Growth Method for the Study of Vitamins" - Jour. Am. Chem. Soc., Vol. LV, p. 2719.,Pub. 1923, Easton, Pa., U.S.A.

washed as before with 500 c.c. of 60% alcohol and finally with 500 c.c. of 90% (by weight) alcohol to facilitate subsequent air drying."

(b) Quantitative Relations of Gains in Body Weights of Experimental Animals to Relative Amounts of vitamin B fed (1).

The average results of feeding different graded allowance of vitamin B are shown in the weight curves Figure III, Series I (2).

"On the lower allowances, some of the animals died during the experimental period, so that the latter portions of the curves designated as 3 c.c. and 4 c.c. Series I of Figure III which represent the average mark of the survivors - which were doubtless somewhat more vigorous than the average of all. Series II shows a composite curve of 65 cases, showing average gains made by rats receiving the dosage of vitamin B which is probably best adapted to its quantitative measurement. Below is a chart showing the average gm. of basal diet eaten per week by the 65 rats.

In order that practically all of the animals submitted to experiment may be represented in the final average result, while at the same time the growth is so restricted that any increase intake of the vitamin will certainly be reflected in a greater gain in weight, it seems probable that a rate of

(1) MacArthur E.H. and Sherman H.C. - "A Quantitative Study of the Determination of Vitamin B" - Jour. of Biol. Chem., Vol. 74, p. 107, Pub. 1927, Balt., Md., U.S.A.

(2) From Figure 1, Series I and Series II, Page 109 of above reference (1).

gain such as that resulting from the feedings of 5 c.c. in the experiments represented by Fig. III represents the best level at which to make quantitative comparisons. A large number of additional experiments was, therefore, made at this level of vitamin B intake with the average results shown in Series II of Fig. III. It is noticed that with the intake of vitamin B restricted to the level here proposed as best suited to quantitative comparisons (and with a basal diet excellently adapted to all other nutritional needs), there is first a distinct gain in weight for 2 to 3 weeks, then a slight decline for a similar period during which that weight remains constant. Such smoothness of the weight curve is to be expected in averages of experiments sufficiently numerous to minimize the effects of individual variability.

The experimenter must also be prepared to meet occasional differences in the response of the weight curve to graded allowances of the vitamin according to the nature of the material fed, which may affect the animal's appetite or capacity of the basal diet."

"Figures 4 (1) and 5 (2) represent differences in (weight curve) response to largely increased dosages of food of differnt type, and serve further to emphasize the desirability of working at relatively low levels in order to decrease differences due to such causes and to give to the estimate of relative amounts of the vitamin in different materials as high

(1) (2) From Figure II and III
MacArthur E.H. and Sherman H.C. - "A Quantitative Study of the Determination of Vitamin B" - Jour. of Biol. Chem., Vol. 74, p.110 and 111, Pub. 1927, Balt., Md., U.S.A.

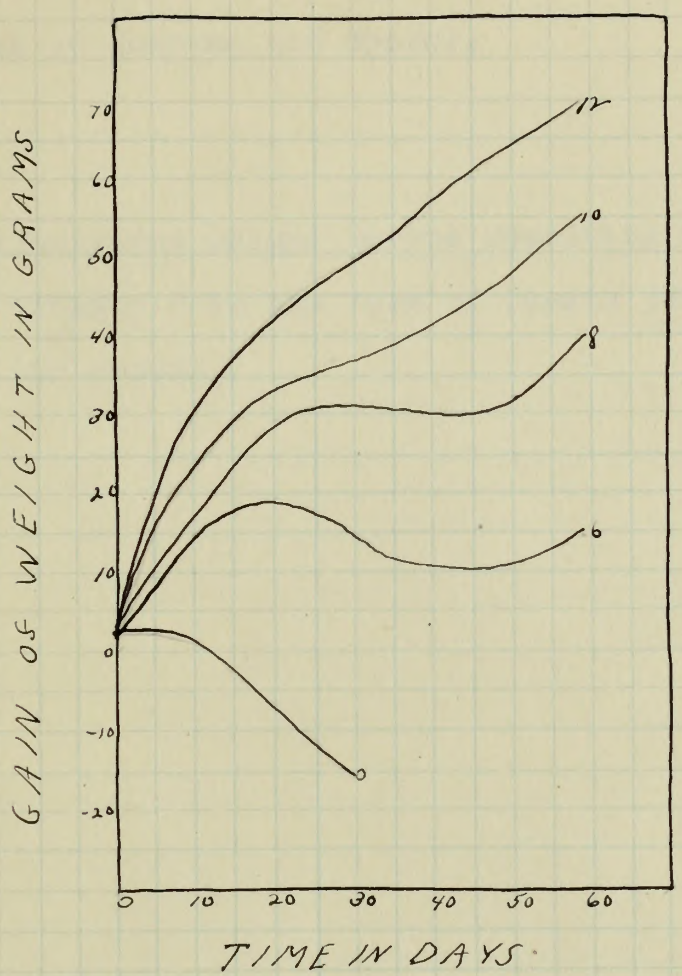

FIG IV(1)

(1) From Fig. II Page 110
MacArthur E.H. and Sherman H.C. - "A Quantitative Study of the Determination of Vitamin B",- Jour.of Biol.Chem.,Vol.74 Pub. 1927, Balt., Md.,U.S.A.

Explanation of:

Figure IV illustrates weight curves resulting from different intakes of vitamin B in the form of skimmed milk powder (experiments of Sherman and Spolm).

Figure V illustrates weight curves resulting from different intakes of vitamin B in the form of tomato juice (experiments of Sherman and Grose).

(b) (Cont)
a degree of quantitative accuracy as possible. For the smaller the amount of material that need be fed to test its vitamin value, the less is the danger that its other constituents or its physical properties may have an appreciable influence upon the results of the experiment."

(c) Length of the Experimental Period

"The weekly weighings of 65 experimental animals fed at the level of vitamin B intake recommended above, have been averaged, and the coefficient of variation and probable error of average weight for each weekly weighing have been computed. There is no significant difference in the probable errors of the means or in the variability of the results such as would in itself serve as a guide to the best length of experimental period; but the general bearing of the data of these and other experiments and the typical form of the weight curves as explained above, confirm the view that an experimental period of 8 weeks is somewhat preferable to a shorter period, such as 6 weeks."

(d) Influence of a Preliminary Depletion Period

"Steenbock, Sill, and Jones, found no evidence of any significant ability to store vitamin B in young rats from 3 to 6 weeks of age (1). Osborne and Mendel (2) have, however, shown definitely a difference in vitamin B content of the livers of

(1) Steenbock H., Sill M.T. and Jones J.H. - "Storage of Vitamin B by the Rat"- Jour. Biol. Chem., Vol.55, p. 411, Pub. 1923, Balt., Md., U.S.A.

(2) Osborne T.B. and Mendell L.B. - "The Effect of Diet on the Content of Vitamin B in the Liver"- Jour. Biol. Chem., Vol.58, p. 363, Pub. 1924-23, Balt., Md., U.S.A.

animals according as they have been fed a normal diet or one lacking this vitamin; in the case of vitamin A,it is now known to be extremely important that the body of the experimental animal be depleted of its surplus before quantitative feeding experiments are begun. Therefore, it seemed desirable to test the effect of a preliminary depletion period in experiments for the measurement of relative amounts of vitamin B. 21 careful comparisons have been made, in each of which,1 rat of a litter (from a previous diet of whole wheat and whole milk) was subjected to a preliminary depletion period by being kept for 10 days or until gain in weight had ceased upon the basal diet, then fed the limited amount of the food for the determination of vitamin B, while a twin of the same sex and initial weight was fed without the depletion period. The uniformity of the results was not enhanced, nor was any other advantage found to result from the interposition of the depletion period, and it may, therefore, be concluded that, important as may be the depletion period in vitamin A, it is no advantage in the case of B, according to conditions in the previous experiments."

(e) Influence of Sex and of Initial Weight upon Results of Feeding a Constant Allowance of Vitamin B (1)

"Osborne and Mendel (2) found that the amount of

(1) MacArthur E.H. and Sherman H.C. - "A Quantitative Study of the Determination of Vitamin B" - Jour. Biol.Chem.Vol.74, Pub. 1927, Balt., Md., U.S.A.

(2) Osborne T.B. and Mendell L.B. - "Quantitative Aspects of the Role of Vitamin B in Nutrition" - Jour. Biol. Chem., Vol. 54,p.739 , Pub. 1922, Balt., Md., U.S.A.

vitamin B required is approximately proportional to the size of the experimental animal (rats). The larger animals were also older. If differences in size or in sex among experimental animals of the same age influence their vitamin B requirements, these factors are to be taken into account in quantitative work upon vitamin B bby the rat-feeding method.

The records of the animals most directly comparable in all other respects were taken; the sexes separated and divided into 4 groups according to initial weight (i.e. body weight at 4 weeks of age when the experimental feeding was begun) and averaged the initial weights and gains in weight with the results shown in Table II."

COMPARISON OF INITIAL WEIGHTS AND AVERAGE GAINS OF MALES AND FEMALES

Group No. / Males	Number of Cases	Average Initial Weight	Average gain for 8 weeks
		gm.	gm.
I	10	34.10	18.80
II	12	44.17	12.08
III	12	54.58	10.25
IV	13	67.38	7.77
Females			
I	9	36.78	17.11
II	9	50.33	7.00
III	9	53.99	8.33
IV	9	64.44	1.67

Table II (1)

(1) From Table I, Page 113.
MacArthur E.H. and Sherman H.C. - " A Quantitative Study of the Determination of Vitamin B" - Jour. of Biol. Chem., Vol. 74, Pub. 1927, Balt., Md., U.S.A.

"It will be noticed that the average gain in weight for 8 weeks for the males was greater than that of the females for a corresponding group, and that,both among the males and among the females, increasing initial weights have resulted in smaller average gains during the 8 weeks of the experimental period, with uniform per capita allowances of vitamin B.

All available cases regardless of sex were divided into 4 groups, according to initial weights, and the average weight curves plotted, as shown in Figure 6.

Therefore, it seems to be established for rats of the same age, as previously shown by Osborne and Mendel for rats of different ages, that the larger invididuals have higher vitamin B requirements, therefore make smaller gains in weight if upon complete deprivation of vitamin B, the larger animals survive, on the average, fully as long as the smaller ones of the same age. When the records of all comparable cases were divided according to sex, it appeared that the average gain for the 8 weeks experimental period was: for 46 males, 11.32 gm.; for 38 females, 8.47 gm.. The females, although weighing slightly less, have a slightly higher vitamin B requirement, and therefore, make slightly less gain upon the same limited intake of the vitamin. The difference, in this case, is less than twice its probable error and may perhaps be accidental."

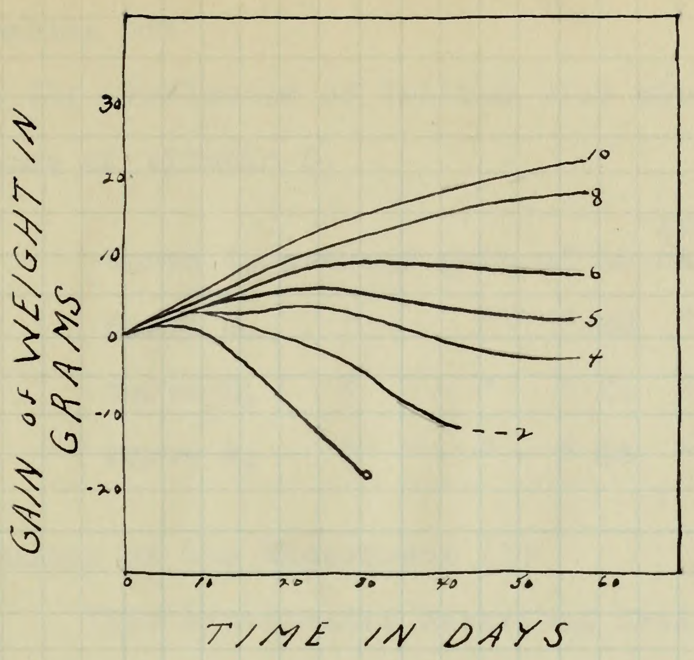

Fig V (1)

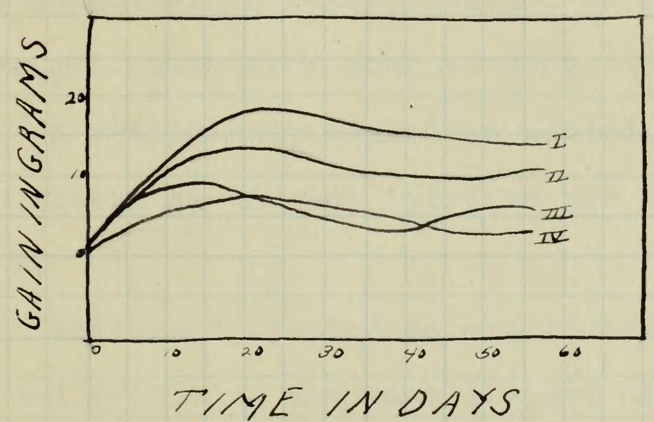

Fig VI (2)

(1) and (2) From Figures III and IV, Pages 111 and 114 MacArthur, E.H. and Sherman, H.C. - "A Quantitative Study of the Determination of Vitamin B" - Jour.of Biol.Chem.,Vol.74, Pub. 1927, Balt., Md., U.S.A.

Explanation of:

Figure VI: Influence of initial size upon response to fixed allowance of vitamin B.

	Average initial weight
Curve 1, average gain of 19 rats	34.63
Curve 2, " " " 22 "	45.77
Curve 3, " " " 21 "	54.29
Curve 4, " " " 23 "	66.74

(f) Summary of the Experiment (1)

"The experiments described deal with several of the factors involved in attempts to measure relative amounts of vitamin B by the rat growth method with as high a degree of quantitative accuracy as is possible.

The levels of feeding vitamin B most conducive to accurate quantitative interpretation of results and the forms of the weight curve from week to week, are critically considered.

A preliminary depletion period, so important in the case of vitamin A, did not increase the accuracy of the work with vitamin B.

Animals subjected to experiments at the uniform age of 4 weeks and receiving the same limited allowance of vitamin B, respond somewhat differently according to their size at the beginning of the experimental period, the larger animals making the smaller gains."

(1) MacArthur E.H. and Sherman H.C. - "A Quantitative Study of the Determination of Vitamin B" - Jour. of Biol. Chem., Vol. 74, Pub. 1927, Balt., Md., U.S.A.

"Under like conditions, males seem to make slightly larger gains than females, indicating that the latter have a somewhat higher vitamin B requirement, at least when of equal size; but the difference, between the average for males and females, was less than twice its probable error and may probably have been accidental."

Chapter VI

The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A) (1) (2)

(a) Introduction

"The comparable influence on rickets of cod-liver oil, sunlight, and the mercury-vapour quartz lamp is now a well-established fact, both in the case of infants and in the case of rats. The antirachitic factor in cod-liver oil has been much discussed, and its identity with the fat-soluble A factor is definitely denied by McCollum (3) and his co-workers, who retain the name, fat-soluble A or vitamin A for the factor which prevents xerophthalmia and promotes growth in rats receiving an otherwise complete diet. Further evidence in regard to this matter is badly wanted.

The present experiments were the outcome of work on rickets in infants conducted in Vienna in conjunction with a number of other workers, (Chick, Dalyell, Hume, Mackay and Henderson Smith, 1922) (4). The experiments were devised to ascertain whether any such interaction or mutual substitutability exists between vitamin A and light for the growth of rats, as

(1) Hume E.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A),"The Lancet, Vol.II, p.1318, Pub. 1922, London, Eng.
(2) Goldblatt H. and Soames K.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in the fat-soluble growth-promoting factor)," The Lancet, Vol.II, p.1321, Pub. 1922, London, Eng.
(3) McCollum E.V., Simmonds N., Shipley P.G., and Park E.A.-"Is There a Substance Other than Fat-Soluble A Associated with Certain Fats which Plays an Important Role in Bone Development?" Jour. Biol. Chem., Pub. 1922, Balt., Md., U.S.A.
(4) Chick H., Dalyell E.J., Hume E.M., Mackey M.M. and Smith H.H. "The Aetiology of Rickets in Infants",-The Lancet, Vol.II, p.7, Pub. 1922, London, Eng.

has been demonstrated to exist between a vitamin factor and light for rickets. If such an interaction were found to exist, the probability of the identity of the antirachitic factor with vitamin A would be heightened. It was also hoped to throw some light on the nature of the reaction should it be found to exist."

"McCollum (1) and his co-workers observed in the course of their experiments on the prevention of rickets in rats by irradiation with the mercury-vapour quartz lamp that there was a slightly greater weight increase in the rayed animals (average weight at the end of experiment - 157 gm.) than in the controls (average 140 gm.), and that the former ate better, were more active and were in a better condition generally."

"Eckstein (2), at the meeting of the Deutsche Gesellschaft für Kinderheilkunde, Leipzig, Sept., 1922, narrated some observations on the influence of the mercury-vapour quartz lamp on the growth of young rats fed on a vitamin-free diet (The details of the experiment are not yet published). In the course of the discussion on Eckstein's paper, the main points of the experiment about to be described were communicated on the writer's behalf by Dr. R. Wagner. Otherwise there do not appear to be any previous observations dealing with the subject."

(1) McCollum E.V., Simmonds N., Shipley P.G., and Park E.A.-"Is There a Substance Other than Fat-Soluble A Associated with Certain Fats which Plays an Important Rolê in Bone Development?" Jour. Biol. Chem., Pub. 1922, Balt., Md., U.S.A.

(2) HumeE.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)," The Lancet, Vol.ii, p. 1318, Pub. 1922, London, Eng.

(b) Experimental Procedure.

The experiment is comprised as follows (1):

"Group 1. Systematic irradiation of rats fed on a diet deficient in Vitamin A, irradiation and deficient diet commencing from the same date.

Group 2. Systematic irradiation of rats, long fed on a diet deficient in vitamin A which had begun to develop eye symptoms.

Group 3. Systematic irradiation of rats which had been for shorter periods previously on a diet deficient in vitamin A.

158 days on deficiency diet, 44 days ray treatment, 202 days duration of experiment.

The diet used had the following composition: Heated caseinogen, 180 gm., maize starch, 520 gm., hardened cotton-seed oil, 150 gm., salt mixture, 50 gm., H_2O, 600 gm.

The food was in excess of the amount required by each animal each day, and the residues were collected every second day. The rats were kept singly in eight litre-glass jars in a north room; they were bedded with saw-dust and had a wooden house. Water was supplied ad libitum.

Rats subjected to the rays at 80 cm. were held in the hand, and the head and eyes were shielded by the hand or with black paper. The dose first used was as much as 20 minutes daily, attained by several steps, but later 10 minutes every second day was used consistently. No attempt was made to ascertain a minimum dose."

(1) Hume E.M.- "The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol.ii, p. 1318, Pub. 1922, London, Eng.

(c) Experimental Details

"Group 1. Systematic Irradiation of Rats on a Diet Deficient in Vitamin A (1) - Irradiation and deficient diet commence from the same date. Three rats (Nos. 151, 155, 158, Fig. 7) belonging to three separate families of three were selected for the experiment, and three other rats, each belonging to one of the same three families were used as controls (Nos. 153, 156, 159 Fig. 7). Owing to the great difference in susceptibility to a vitamin - A deficiency (Korenchevsky, 1921) (2) known to exist between rats of different rearing, it is of the greatest importance that rats in experiments, such as these, should have controls from the same litters. At a weight between 50 to 60 gm., both experimental rats and controls were placed upon the diet deficient in vitamin A, at the same time irradiation of the experimental rats was begun. In one case, two initial exposures were for five minutes, and in two cases, only one preliminary exposure of five minutes was given. Subsequently,raying took place for ten minutes on every second day. The rayed rats grew at a rate well above the normal, which is, however, admittedly rather low, for a period varying from 35 to 50 days. Their curve of growth then began to flatten off, and soon showed actual decline. Two had rhinitis from the 60th day. All three became intensely excitable and nervous, a symptom noted between the 50 and 60th days. The first symptoms of xerophthalmia, slight secretion and slight enophthalmos appeared between the ----------------------

(1) Hume E.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol.ii, p.1318, Pub. 1922, London, Eng.
(2) Korenchevsky H.-"Experimental Rickets in Rats"- British Med. Jour., Vol.ii, p. 754, Pub. 1921, London, England.

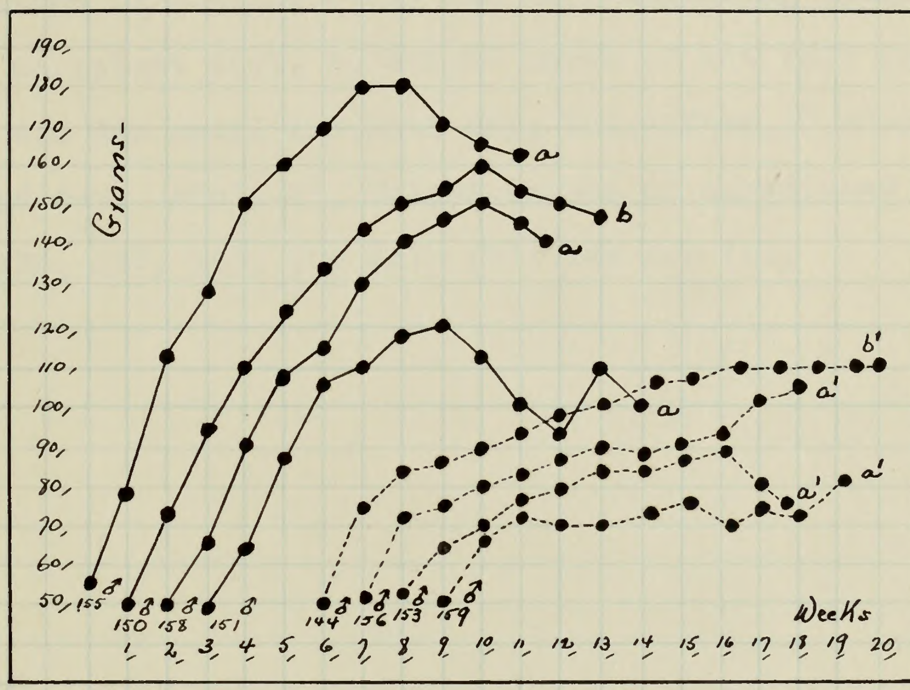

Fig. VII (1)

(1) From Page 1320, Chart I.
Hume E.M. -"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol.ii, p.1318, Pub. 1922, London, Eng.

Explanation of:

Figure VII(1). Influence of radiation from mercury-vapour-quartz lamp upon growth of rats upon a diet deficient in vitamin A. a a, Weight curves of rats 151, 155, 158; irradiation 10 minutes every 2nd day, beginning simultaneously with deficient diet. b, Weight curve of rat 150; irradiation from 6th day until the 30th day, thereafter,10 minutes every second day. a'a', b', Weight curves of unradiated controls (a'),153, 156, 159, for (a); (b') 144 for (b).

(1) From Page 1320, Chart I.
Hume E.M. - "The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol.ii, p.1318, Pub. 1922, London, Eng.

50th and 60th days. More severe symptoms, corneal dryness, and opacity soon followed some 10 days later. Actual ulcers occurred ultimately in two out of the three cases. General condition remained for some time superior to that usually seen in animals with such severe eye symptoms. One animal (No. 151) died on the 77th day without any symptoms other than those of severe vitamin A deficiency. One (No. 158) was removed from the experiment on the 67th day with severe corneal ulcers, and the experiment was terminated in the case of the third (No. 155) on the 77th day, when corneal ulcers were also present. The three irradiated animals, therefore, showed a period of normal growth of from 35 to 50 days' duration, followed by the onset of typical symptoms of vitamin A-deficiency, to which was added a condition of great nervous excitability not usually observed. The controls (Nos. 153,156, 159, Fig.7) grew normally for a period of from 7 to 10 days, then their curve of growth flattened off, showing continuous slight growth, far below the normal, for a period, in the case of two, of 77 days, when the experiment was terminated. The 3rd animal refused to eat the synthetic diet after the 66th day and lost weight severely; cod-liver oil was also added to the diet, but this was also refused. Recovery took place on bread and milk, however, and these facts, coupled with the complete lack of symptoms, suggested that the animal's decline was not directly due to lack of vitamin A, but to a simple refusal to eat anything at all of the synthetic diet. The general condition of the three controls continued to be very fair, although they remained so small. One animal was rather excessively nervous, and one had slight rhinitis. As regards the eyes, no animal had

by the 77th day showed more than slight symptoms of enophthalmos. The rayed animals, therefore, grew far in excess of the unrayed animals, normal growth being maintainted for about 5 times as long. After the prolonged period of normal growth, however, the rayed animals developed the typical symptoms of vitamin A-deficiency with greater severity and greater rapidity than did the control animals; this is particularly true of the symptoms of xerophthalmia, the rayed rats already showed corneal alterations (in two cases ulceration) when the controls only showed early signs of enophthalmos. Conclusions drawn from the date of onset of xerophthalmia in so small a series of rats would not usually be accepted, but the difference in this case is so great and the uniformity so complete that it does seem possible to draw a conclusion.

A fourth rayed animal (Fig. 7, No. 150) and its control (Fig. 7, No. 144) have not been included in the foregoing series owing to the different dose of irradiation given. Irradiation did not begin until the sixth day of deficient diet, when a dose of twenty minutes every second day was quickly reached by steps and continued till the 30th day, when the dose was reduced to 10 minutes every 2nd day. The history does not, however, materially differ; normal growth continued in the rayed animal till about the 50th day. About the 30th day, slight eye symptoms appeared, but retrogressed completely, and did not reappear till about the 70th day, from which time they became progressively more severe.

Table III (1)

Table Illustrating Treatment with the Mercury-Vapour Quartz Lamp of Rats previously on a Diet Deficient in Vitamin A for Varying Periods and Time

No. of Rat	No. of da. on def. diet before raying starts	Presence of eye symptoms: Before raying	Presence of eye symptoms: After raying	Exposure to rays at 80 cm. in minutes	Duration of treat. in days	Wt. gm. commencement (1)	Wt. gm. end (2) of raying	Remarks
88	144	+	No improvement	20 daily - thru glass 10 every 2nd day-no "	14 7 21	125	92	Died
110	158	+	Worse	10 every 2nd day	24	106	104	--
131	45	+	Worse with marked temp. improvement	20 - 30 daily 10 every 2nd day	28 13 41	104	100	Wt. rose to 122gm. on 21st day
132	115	+	Worse	10 every 2nd day	19	119	110	--
133	115	+	No improvement	10 every 2nd day	17	126	109	--
169	91	+	Worse	10 every 2nd day	6	120	110	Very ill
170	91	+	Worse	10 every 2nd day	19	129	110	Died
171	91	Very slight	Worse	10 every 2nd day	24	129	107	Died

(1) From Page 1319, Table I
Hume E.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)," The Lancet, Vol.ii, Pub. 1922, London, Eng.

The experiment was terminated on the 97th day, when the eyes showed deep enophthalmos with much secretion and frequently adhering lids but no ulceration of the cornea. Rhinitis was present. The control, No. 144, Fig. 7 (a brother of 150), grew normally for about 14 days, showed slight enophthalmos on the 84th day and dryness of the cornea on the 100th day, when the experiment was terminated; on that date the animal had an attack of acute tetany, from which it recovered; its general condition was fair. The result with these two animals thus falls into line with the results already detailed."

"Group 2. Systematic Irradiation of Rats long Fed on a Diet Deficient in Vitamin A, and Beginning to Develop Eye Symptoms (1). - The animals used in this experiment agreed in having been for long periods of time on the same vitamin A deficient diet as was used for the experiments in Group 1, and in having been long stationary in growth. Two (Nos. 88 and 110 in table 3) had, however, received, for an intermediate part of the period a small ration of milk insufficient to induce more than slight growth; they had been without this ration, one for 35 and one for 64 days, before irradiation began. The table gives the particulars of 8 such rats, most of which were irradiated for 10 minutes every second day, but of which one (No. 131) was irradiated for 20 to 30 minutes daily for 28 days, and another (no. 88) for 20 minutes daily, but through glass for 14 days. It is clear from the table that when irradiation was thus

(1) Hume E.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)," The Lancet, Vol.II, p. 1318, Pub. 1922, London, Eng.

the experiment was terminated on the 77th day, when the eyes showed deep xerophthalmia with much secretion and frequently sneezing like but no ulceration of the cornea. Keratitis was present. The control, No. 148, Fig. 7 (a brother of 150), grew normally for about 15 days, showed slight xerophthalmia on the 84th day and signs of the cornea on the 100th day, when the experiment was terminated; on that date the animal had an attack of acute tetany, from which it recovered; its general condition was fair. The result with these two animals thus falls into line with the results already detailed."

"Group 2. Systematic Irradiation of Rats long fed on a Diet Deficient in Vitamin A, and beginning to Develop the Symptoms (1). - The animals used in this experiment agreed in having been for long periods of time on the same vitamin A deficient diet as was used for the experiments in Group 1, and in having been long stationary in growth. Two (Nos. 88 and 110 in table 3) had, however, received, for an intermediate part of the period a small ration of milk insufficient to induce more than slight growth; they had been without this ration, one for 36 and one for 64 days, before irradiation began. The table gives the particulars of 8 such rats, most of which were irradiated for 20 minutes every second day, out of which one (No. 121) was irradiated for 20 to 30 minutes daily for 28 days, and another (No. 80) for 20 minutes daily, but through glass for 14 days. It is clear from the table that when irradiation was thus

(1) Hume E.M. - "The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol.II, p. 1318, Dec. 1922, London, Eng.

applied for periods varying from 6 to 24 days to rats which had been on a deficient diet already for from 91 to 158 days, there was no resumption of growth, but rather loss of weight, symptoms of xerophthalmia failed to improve or get worse, and death occurred in 3 cases. In one case (No. 131) there was a temporary resumption of growth (18 gm.in 22 days) and retrogression of eye symptoms, but the improvement was only maintained for 21 days, although irradiation was continued for, in all, 41 days. A similar temporary improvement is sometimes observed in controls, but it is noteworthy that this animal had been for a shorter period than most (only 45 days) on a deficient diet before irradiation began and the observations made on the next group make it probable that it is with that fact that the temporary recovery is to be correlated. It would, therefore, appear that rats which have been for long (90 days and over) on a diet deficient in vitamin A cannot be recovered either as regards growth or xerophthalmia, by irradiation with the mercury-vapour quartz lamp."

"Group 3. Systematic Irradiation of Rats Fed for Varying Shorter Periods on a Diet Deficient in Vitamin A (1). The procedure was the same as in the foregoing groups, except that irradiation was applied for 10 minutes daily instead of every other day, a fact which is not thought in any way to damage the comparison. The eyes were not shaded from the lamp;

(1) Hume E.M.-"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol.ii, p. 1318, Pub. 1922, London, Eng.

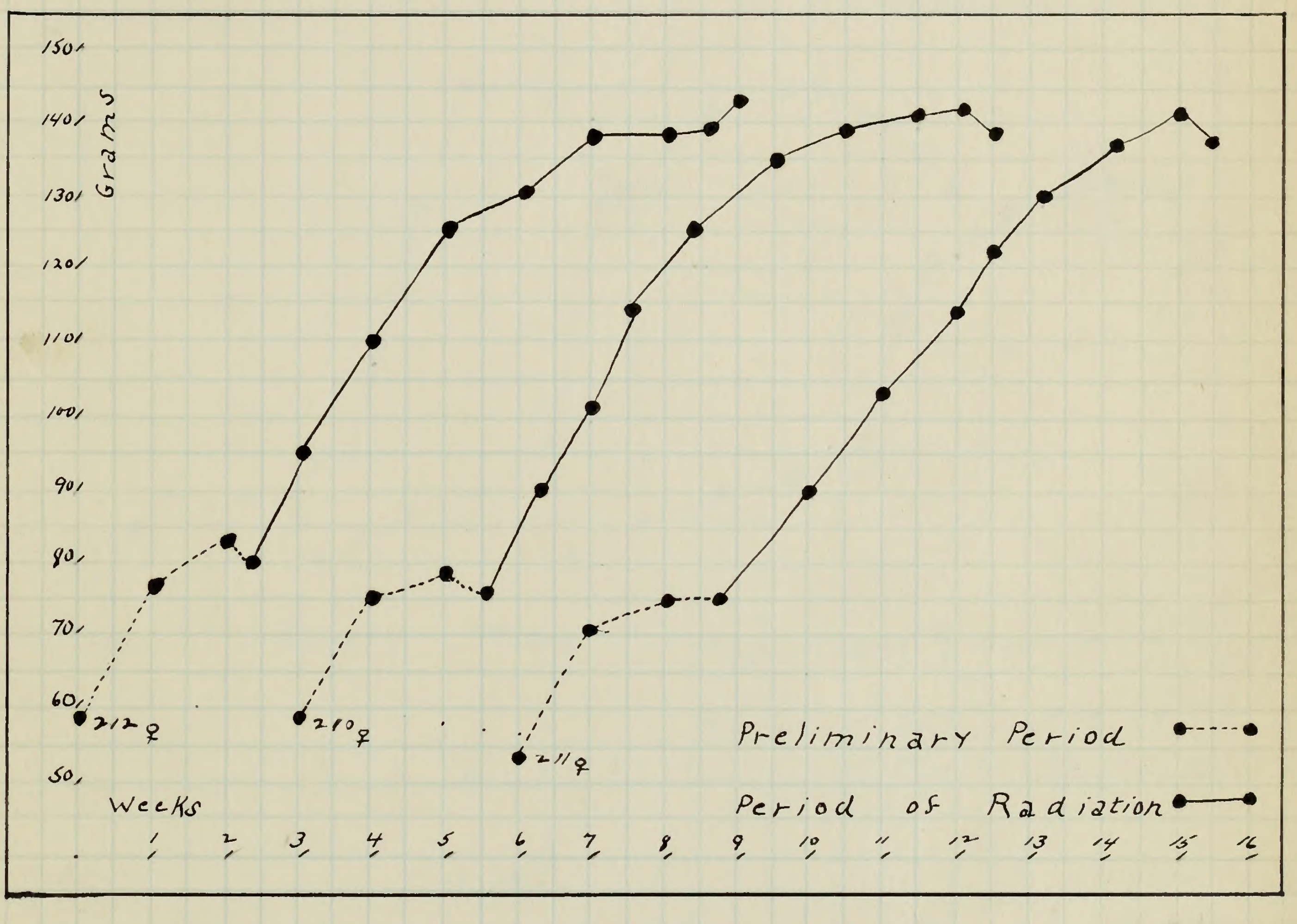

Fig VIII [1]

(1) From Page 1320, Chart II.
Hume E.M. -"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)," The Lancet, Vol. II, p.1318, Pub.1922, London, Eng.

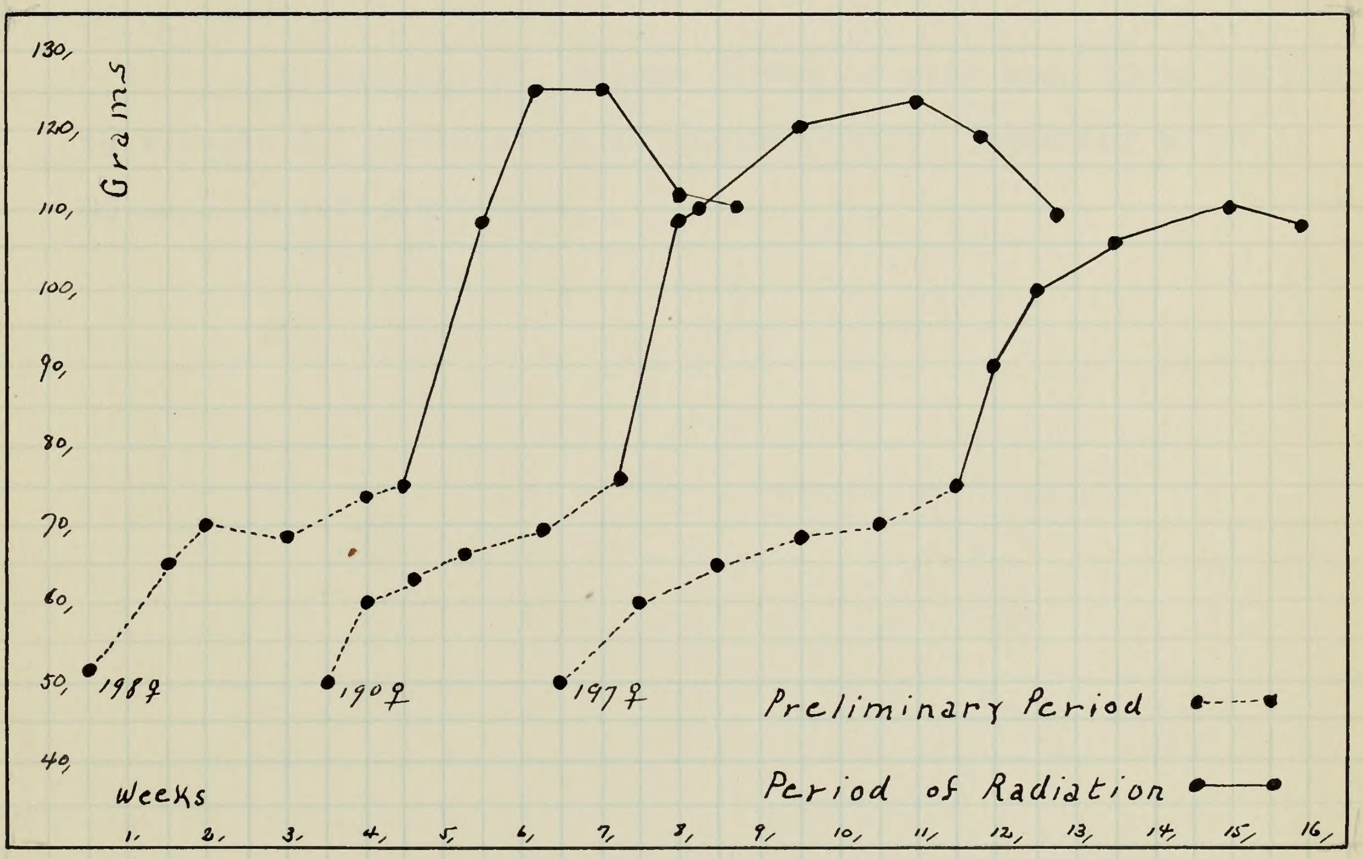

Fig IX (1)

(1) From Page 1321, Chart III.
Hume E.M. -"The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol. II, p. 1318, Pub. 1922, London, Eng.

Explanation of:

Figure VIII. (1). Weight curves of rats upon diets deficient in vitamin A. Irradiation 10 minutes daily, beginning after 17 days on deficient diet.

Figure IX.(2). Weight curves of rats upon diets deficient in vitamin A. Irradiation 10 minutes daily, beginning after 35 days on deficient diet.

(1)Page 1320, Chart II, (2) Page 1321, Chart III.
Hume E.M.- "The Effect of Radiation with the Mercury-Vapour Quartz Lamp (on the growth of rats fed on a diet deficient in Vitamin A)", The Lancet, Vol. ii, p.1318, Pub. 1922, London, Eng.

hence no conclusions were drawn with regard to xerophthalmia. Fig. 8 shows the curves of 3 rats with which irradiation was begun on the 17th day of deficient diet, when normal growth had already ceased for about 10 days. The weight curve at once again lifted to the normal rate and growth was still continuing, though not quite normally, on the 56th day, when all 3 rats had had a weight of 140 gm. Fig. 9 shows the curves of 3 rats in which irradiation was begun on the 35th day of deficient diet. The response was the same as in the preceding experiment, but it was not maintained for so long, about 14 days as against about 28 days, and the maximum weight attained was not as great, about 125 gm. as against 140 gm. and over. Irradiation was not attempted at periods intermediate between 35 days and the times described under group 2, but it can hardly be doubted that the duration and extent of the response to irradiation must be roughly inversely proportional to the length of the previous period on vitamin A-free diet, all other conditions being equal."

(d) Summary of the Experiment

"From each group experiment it was found that irradiation with the mercury vapour quartz lamp can prolong normal growth on a diet almost free, or free, from vitamin A (1). Whereas, without irradiation, growth ceased to be normal after a period of from 7-10 days, with irradiation, it continued to be normal from 35 - 50 days. Symptoms of vitamin A-deficiency then began to appear and symptoms of xerophthalmia set in earlier than in control animals.

(1) Hume E.M.- "The Effect of Radiation with the Mercury-Vapour Quartz lamp (on the growth of rats fed on a diet deficient in Vitamin A)" The Lancet, Vol.ii,p.1318, Pub.1922, London, Eng.

"Attempts to revive growth and to cure xerophthalmia by irradiation of animals which had long (over 90 days) been on a deficient diet failed completely; the animals seemed, if possible, to go to pieces more rapidly.

Irradiation of rats which had been for shorter periods (17 - 35 days) on a vitamin A-deficient diet produced a growth response which proved to be inversely proportional in its duration and extent, to the length of the previous period of deficient diet.

The general conclusion drawn is that there is an interaction between vitamin A and light for the growth of rats, but that the action of light is not to produce a photosynthesis of the vitamin. It would appear that on cessation from normal diet that there is a larger or smaller store of vitamin A in the body which is for a few days so available that normal growth continues. Very soon, however, it ceases to be thus available, growth almost, but not quite ceases, and the vitamin store serves the animal for maintenance for a long period of weeks. At length, the store is quite used up and bankruptcy can no longer be prevented, the animal entirely ceases to grow, usually develops severe xerophthalmia, loses weight, and dies. The early cessation of normal growth, followed by a long period of maintenance, might almost be regarded as a defense mechanism, which enables the rat by minimum expenditure of vitamin A to tide over a period of vitamin A famine, which might easily be a danger in the normal life of the rat.

When irradiation is applied it would seem as if either the low level of vitamin A metabolism is rendered sufficient."

That is,the vitamin is economized - or else the vitamin stores are forced to yield up sufficient vitamin A at a rate which suffices to produce normal growth until the supplies are wholly exhausted. This effect can apparently be produced at any time before the end of the period of slow growth and the amount of normal growth produced as a result would appear to be inversely proportional to the length of time any individual rat has previously been on the vitamin A-deficient diet. Ultimately, when presumably all stores have been exhausted no normal growth can be evolved by irradiation. Hence, it is clear that the light can neither create or be substituted for the vitamin, it appears to act only as an economizer, or activator when it is already present. Whether the interplay shown to exist between vitamin A and light for the growth of rats is wholly similar to that shown to exist between a vitamin factor and light for rickets is not possible to say at present."

"Examination of the results obtained by McCollum (1) and his co-workers in preventing rickets in rats with sunlight and with ultra-violet light shows that the animals were on the deficient diet only 62 - 67 days, and 64 days respectively, and the diets are only characterized as below the optimum in vitamin A. The experiment did not last long enough to determine if the protection was permanent or not, and the supply of vitamin A factor was not nil. The same applies to experiments of Hess, Unger, and Pappenheimer (2); the diet was lower in vitamin A, but the experimental work lasted only 30 - 40 days.

(1) Hume E.M.-"The Effect of Radiation with the Mercury-Vapour Quartz lamp (on the growth of rats fed on a diet deficient in Vitamin A)" The Lancet, Vol.ii, p.1318, Pub. 1922, London, Eng.

(2) Hess A.L., Unger L.J., Pappenheimer A.M.-"The Prevention of Rickets in Rats by Exposure to Sunlight"- Jour. Biol. Chem., Vol. L, p. 77, Pub. Balt., Md., U.S.A.

The existing experimental evidence does not, therefore, exclude the possibility that the interplay in growth and rickets is the same, and the very existence of an interplay in both cases heightens the possibility that the two vitamin factors may be identical or nearly allied."

Chapter VII

The Metabolism of Vitamins

(a) Distribution of Vitamins in Food

"From (1) the standpoint of practical dietetics, it is of great importance to determine the vitamin content of the more commonly used foodstuffs.

The available data bearing on this point were obtained by means of feeding experiments on rats, guinea-pigs, pigeons, and chickens. To a basal diet, complete in every respect but lacking the vitamin under consideration, there were added the foodstuffs to be investigated in such amounts as to just maintain nutrition or growth. The results, although not absolutely accurate, may be summarized as follows: The principal sources of the antineuritic vitamin are the green parts of plants, the seeds of plants, eggs, animal tissue, with exception of adipose tissue, pulses, and to a more limited extent, milk and fruits. Brewer's yeast is very rich in this factor. In the case of cereals this factor is located within or close to the embryo, which accounts for the deficiency of the highly milled products in this factor, as the milling process removes the embryo and the superficial layers of the seed.

An important relationship between the dietary value of the natural foods was brought out by the systematic investiga-

(1) Voegtlin, C.-"Metabolism of Vitamins" - "Endocrinology and Metabolism," Vol. 3, p. 341, Pub. 1922, London, Eng.

tions of McCollum and his co-workers (1917), who were able to show that the addition of the green parts of the plants to a diet restricted to the seeds of plants has a marked tendency to render the diet more complete, not only in respect to the inorganic salts, but also, the fat-soluble vitamin, and previous work had shown that green vegetables supply, furthermore, the antiscorbutic vitamin which is absent in cereals. The conclusion is, therefore, justified that a proper mixture of the green parts of plants and the seeds does possess a higher dietary value than either of these foodstuffs alone. A mixed diet containing in addition to cereals and green vegetables, also milk and fresh meat is the best safeguard against the possibility of a vitamin deficiency and furthermore insures an adequate supply of inorganic salts and protein of proper biologic value."

(b) Digestion and Absorption of Vitamins (2)

"Due to the relatively unstable character of the vitamins, it is a matter of importance to know whether these substances are partly destroyed during digestion. Quantitative information is entirely lacking on this point. However, it may safely be assumed that the utilization of vitamins contained in certain foods (yeast, butter, lemon juice) is fairly efficient, as very small quantities of the latter are required to supply the animal's needs in vitamins. Whether vitamins are absorbed by the stomach or the upper intestine, or both of these organs, remains to be determined."

(1)McCollum E.V., Simmonds N., Pitz W.-"The Supplementary Dietary Relationship between the Leaf and Seed as Contrasted with Combinations of Seed with Seed,"-Jour.Biol.Chem.,Vol.XXX,p.13-32,Pub.1917, Balt., Md., U.S.A.
(2)Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism"-Vol.3,p.341, Pub.1922, London, Eng.

(c) Intermediary Metabolism and Physiological Action (1)

"After absorption from the gastrointestinal canal, the vitamins are carried, presumably, by way of the portal circulation, or possibly also by the lymphatics to the tissues of the body, where they are stored up; it is interesting to note that different organs vary considerably in their vitamin content. Cooper (1913) (2) has shown that the antineuritic vitamin is largest in ox liver, less in ox heart, and still less in ox brains and skeletal muscle, the latter containing only small amounts of the vitamin. The presence of this vitamin was also demonstrated in the spinal cord (Voegtlin and Towles, 1913) (3), the pancreas (Eddy, 1916) (4), the kidney (Osborne and Mendel, 1917 -1918) (5), and testicles (Schaumann,1910) (6), whereas it seems to be absent from adipose tissue generally.

(1) Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism" - Vol. 3, p.341, Pub. 1922, London, Eng.

(2) Cooper E.A.-"The Preparation from Animal Tissues of a Substance which Cures Polyneuritis in Birds Induces by Diets of Polished Rice"-Biochem.Jour., No.XXCII,Pt.I,Vol.VII, pp.268-274, Pub.1913, London, Eng.

(3)VoegtlinC. and Towles C.-"The Treatment of Beri-beri with Extracts of Spinal Cord"-Jour. Pharmacol.,Exp. Ther., Vol.V,p.67-76, Pub.1913, Balt., Md., U.S.A.

(4) Eddy W.H.-"The Isolation of a Growth-Producing Substance from Sheep Pancreas"-Jour. Biol. Chem.,Vol.XXVII, Pub.1916, Balt., Md., U.S.A.

(5)Osborne T.B. and Mendel L.B.-"Nutritive Factors in Animal Tissues" -Jour. Biol. Chem.,Vol.XXXVII,p.309-323,Pub.1917, Balt.,Md., U.S.A.
Osborne T.B. and Mendel L.B.-"Nutritive Factors in Animal Tissues" -Jour. Biol. Chem.,Vol.XXXIV, p.17-20, Pub.1918, Balt., Md., U.S.A.

(6) Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism" - Vol.3, p.341, Pub. 1922, London, Eng.

These observations are rather significant, as they suggest that the antineuritic vitamin is needed in all tissues, more or less in proportion to the magnitude of their metabolism, but not in tissues which function as a depot for reserve energy. This interpretation is also supported by the fact that the yolk of eggs are rich in this vitamin, whereas it seems to be absent in egg white. Likewise, the distribution of this substance in plant tissue, supports this deduction; for it is concentrated within or in the immediate neighborhood of the embryo or germ of the corn and wheat kernel and that it is absent in the superficial layers and endosperm (Voegtlin and Myers 1919) (1). More recent work has also shown that the green parts of plants contain considerable quantities of antineuritic vitamin, when due allowance is made for the high water content of these foods (Osborne and Mendel,1919) (2)."

"A somewhat different situation is met with in the distribution in the body of the fat-soluble vitamin which is found, not only in glandular organs, but also in certain adipose tissue (beef fat). Quite singularly, it is absent from lard, and skeletal muscle seems to contain only traces. Furthermore, the liver is relatively rich in this vitamin, as shown by the high activity of cod-liver oil."

(1)Voegtlin C. and Meyers C.N.-"Distribution of the Antineuritic Vitamin in the Wheat and Corn Kernel,"- Am. Jour. Physiol.,Vol. XLVIII, pp.504-511, Pub. 1919, Boston, Mass., U.S.A.

(2) Osborne T.B. and Mendel L.B.-"The Distribution of Water-Soluble Vitamin"-Jour. Biol. Chem., Vol.XXXIV, p.29-34, Pub. Balt., Md., U.S.A.

"There is almost no available data giving the localization of the antiscorbutic vitamin in the various organs of the body, with the exception of the well established fact that fresh, lean meat contains this substance.

Numerous feeding experiments with deficient diets, leads us to state that the animal body, under normal conditions, contains a considerable reserve of fat-soluble vitamin, but not of antineuritic and antiscorbutic vitamin. Thus, susceptible animals survive a much longer period on a diet containing less of the former, than on a diet deficient in the latter two vitamins.

As regards the role played by vitamins in metabolism, we are still more or less limited to hypothetical considerations supported to some extent by suggestive observations. One of the most perplexing questions is the fact that different species of animals have different vitamin requirements. For example, it has been shown that a diet complete in every respect but completely lacking the antiscorbutic vitamin will support normal metabolism, growth, and maintenance of health in rats, mice, pigeons and chickens for considerable periods, whereas this same diet will cause scurvy within a few weeks in man, guinea-pigs, monkeys and dogs."

"However, it has been shown that all of the higher animals need a certain amount of fat-soluble and antineuritic vitamin for proper nutrition, maintenance of normal growth, reproduction and life. It has been suggested by various students of this subject that the antineuritic vitamin is also linked

with the proper functioning of the nervous system, an assumption which is supported by the fact that in animals fed on a diet deficient in this vitamin there is an occurrence of severe paralytic symptoms and degenerative changes in the nervous system (of animals fed thus). More recently, McGarrison (1) has shown that the nervous system is not the only organ effected by this vitamin deficiency. A few workers have attempted to prove that the antineuritic vitamin has a stimulating action upon various digestive glands, this resulting in an increased production of secretion."

"Uhlman(1918) (2) studied the effect of the residue of an alcoholic extract from rice polishings on the various glands and on the sweat glands. He obtained a marked increase in secretion, following the first injection of the extract. He was, furthermore, able to show that the same extract caused contraction of intestinal muscle and a fall in blood pressure. The latter effect he attributed to a direct depressing effect on the heart muscle and to vasodilation. Voegtlin and Myers (1919) (3) published their findings without a knowledge of Uhlman's work. They showed that an intravenous injection of a highly purified extract from yeast produced an abundant flow of pancreatic and biliary secretion, resembling, in every respect, the effect produced by an extract of the duodenal mucosa purified in the same manner as the yeast extract.

(1) (2) Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism"-Vol.3, p.341, Pub. 1922, London, Eng.

(3) Voegtlin C., and Meyers C.N.-"A Comparison of the Influence of Secretin and Antineuritic Vitamin on Pancreatic Secretion and Bile Flow"-Am. Jour. Physiol.,Vol.XLIX,p.124,Pub.1919,Boston,Mass.,U.S.A.

Alcoholic extracts from liver produced the same effect, and all three extracts were shown to be rich in antineuritic vitamin, when tested as to their therapeutic action on polyneuritic pigeons. As suggestive and interesting as these facts may be, it should be emphasized that the physiological effect noted by all these observers may have been due to some highly active impurity, and not the vitamin, "per se". "

"Dutcher (1918) (1) has suggested some relation between the antineuritic vitamin and oxidation processes, as he observed that the tissues of polyneuritic birds showed a marked reduction in catalase and that the catalase activity was again restored to normal after the administration of this vitamin. He believes that this substance increases the production of catalase."

"Funk (1919) (2), Braddon and Cooper (1914) claim that (3) the antineuritic vitamin is essential for the metabolism of carbohydrates, a theory which is not shared by Vedder (1918) (4)."

"Drummond (1918) (5) studied the metabolism of rats fed on an artificial diet deficient in antineuritic vitamin and noted the presence of creatinuria, accompanied by decrease in food consumption. The addition of the vitamin to the diet was followed by an increased food intake."

(1)Dutcher R.A.-"Observations on the Catalse Activity of Tissues in Avian Polyneuritis"-Jour. Biol.Chem.,Vol.XXXVI,p.63-72, Pub.1918, Balt., Md., U.S.A.

(2)Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism"-Vol.3,p. 341, Pub. 1922, London, Eng.

(3)Braddon W.L. and Cooper E.A.-"The Influence of the Total Fuel Value of a Dietary upon the Quantity of Vitamin Required to Prevent Beri-beri"-British Med.Jour.,Vol.I,p.1348-1349,Pub.1914,Lon.,Eng.

(4)Vedder C.-"Is the Neuritis-Preventing Vitamin Concerned in Carbohydrate Metabolism?"-Jour.Hyg.,Vol.XVII,p.1-9,Pub.1918,London, Eng.

(5) Drummond J.C. "A Study of the Water-Soluble Accessory Growth-Promoting Substance", Biochem.Jour.,Vol.12,p.25,Pub.1918,London,Eng.

"We might mention here the work of Mellanby (1919) (1) who claims to have produced experimental rickets in dogs by means of a diet deficient in the fat-soluble vitamin, which would indicate that the substance is concerned with the metabolism of calcium. This view may be accepted but with considerable modification, for Hess and Unger (1920) (2) have shown conclusively that infants develop rickets, while receiving "a full amount of this principle, and that they do not manifest signs, although deprived of this vitamin for many months, at the most vulnerable period of their life." McCollum and Simmonds (3) have also presented evidence which is not in accord with Mellanby's view."

"A lack of fat-soluble vitamin in the diet leads to the appearance of xerophthalmia in rats, McCollum (4); a condition which previously had, in 1904, been observed by Mori (5) in young children whose diet was lacking in certain fats, which are now known to be rich in fat-soluble vitamin."

"The antiscorbutic vitamin is probably concerned with the growth of some species, but not of all, as Hess (1916) (6) ob-

(1) Mellanby E.-"Experimental Investigation on Rickets" The Lancet, Vol I, p.407, Pub. 1919, London, Eng.

(2) Hess A.F. and Unger L.J.-"The Clinical Role of the Fat-Soluble Vitamin; Its Relation to Rickets"-Jour.Am.Assn.,Vol.LXXIV,p.217-223, Pub.1920, Chicago, Ill., U.S.A.

(3) (4) (5) Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism"-Vol. e,p.341, Pub.1922, London, Eng.

(6) Hess A.F.-"Infantile Scurvy, Its Influence on Growth"-Am. Jour.Dis.Child, Vol. XII, P.152, Pub.1916, Chicago, Ill., U.S.A.

served the appearance of scurvy in infants, in spite of a previous normal growth. Holst and Fröhlich (1) have described great fragility of the bones in guinea-pigs suffering with scurvy which on histological examination was shown to be due to lack of proper calcification. It would thus, appear that the antiscorbutic vitamin has some relation, either direct or indirect, to calcification."

(d)
End Metabolism of Vitamins (2)

"The available evidence regarding the ultimate fate of vitamins in the body does not permit many positive conclusions. The only data on this point are a few observations on the vitamin content of the various secretions and excreta. Muckenfuss (1918) (3) treated saliva, ox bile and human urine with Fuller's earth and fed these samples of Fuller's earth to pigeons showing acute symptoms, as a result of a polished rice diet. Improvement was noticed when the preparation was given in amounts corresponding to 950 to 3,250 c.c. of ox bile, 400 to 1,325 c.c. fresh saliva or, 4,150 to 6,000 c.c. of urine, from which it was concluded that this vitamin is present in comparatively small amounts in saliva, bile, and only in traces in urine."

(1) Holst A. and Fröhlich T.-"Experimental Scurvy"-Jour. Hyg., Vol.VII, p.634-671, 2pl. (col.), Pub.1907, London, Eng.

(2) Voegtlin C.-"Metabolism of Vitamins"-"Endocrinology and Metabolism"-Vol.3, p.341, Pub. 1922, London, Eng.

(3) Muckenfuss A.M.-"Presence of Food Accessories in Urine, Bile and Saliva"-Jour. Am.Chem.Soc.,Vol.XL,p.1606-1611,Pub. 1918, Easton, Pa., U.S.A.

"Cooper (1914) (1) showed that alcoholic extracts of feces of rice-fed hens and bread-and cabbage-fed rabbits relieved the symptoms of polyneuritis pigeons. This would indicate that at least part of this vitamin is excreted with the feces or is contained in the undigested food in the feces.

That the mammary glands secrete six vitamins (A,B, B_1,B_2,C,D) is well established as feeding experiments with fresh unheated milk has shown that this food belongs to the richest source of fat-soluble vitamin, that it contains also some antiscorbutic, antineuritic, antiberi-beri, antipellagric, vitamin, although, the last four factors seem to be present in small amounts.

The evidence thus far points to the destruction of the vitamins within the body, which renders it necessary to constantly replenish the supply through a proper diet. The ultimate source of this supply is the plant, as the animal tissues are unable to produce vitamins."

(1) Cooper E.A.-"Protection and Curative Properties of Certain Foodstuffs against Polyneuritis Induced in Birds by a Diet of Polished Rice,"-II-Jour. Hyg., Vol.XIV, p.12-22, Pub. 1914, London, Eng.

(e) Effect of Heat and Preservation Processes on Vitamins (1)

"A complication about vitamins is that treatment with heat, chemicals, drying and other processes have different effects for each of the three vitamins A, B and C. Table IV, on the following page, illustrates the effect of treatment on these vitamins with various agents."

"Vitamin D; Ergosterol, the precursor of vitamin D (2), is a comparatively stable substance, not affected by any of the above processes. Vitamin D, produced by irradiation of ergosterol, is destroyed by too long irradiation. It is not destroyed by the above processes."

"Factor P-P, vitamin B_2, or G is heat-stable, which makes it evidently a substance of co-ordinate importance with the longer-known vitamins as an essential factor in normal nutrition, and that deprivation or serious shortage of this substance results in widespread injury to the body tissues (3). Conversely, the liberal feeding of this substance may be expected to play a significant part in inducing a better than average nutritive condition, a problem which is being approached from various angles in the experimental work in this laboratory (3) Vitamin B_2, or G is sparingly soluble in alcohol; this is also true of vitamin F."

(1) Plimmer R.H.A. and Plimmer V.G. -"Food,Health,Vitamins"- 3rd Edition, Pub. 1928, London, Eng.

(2) Same as (1).

(3) Sherman H.C. and Sandels M.R.-"Experiments with Reference to the More Heat-Stable Factor of the Vitamin B Group"-Proc.Soc. of Exp. Biol. Med., Vol.26,p.536, Pub. 1928-29,Utica, N.Y., U.S.A.

Table IV (1)

Effect of Treatment with Various Agents on Vitamins A, B, and C

Agent	Vitamin A	Vitamin B	Vitamin C
DRYING			
(a) Exposed to air	Destroyed	Not destroyed	Destroyed
(b) In a vacuum	Not destroyed	Not destroyed	Destroyed, except in certain cases
HEATING			
(a) Exposed to air	Gradually destroyed	Not destroyed	Gradually destroyed
(b) In a vacuum	Not destroyed	Not destroyed	Destroyed, except in certain cases
(c) Alkaline medium	Not destroyed	Destroyed	Destroyed (2)
HEATING UNDER PRESSURE			
(a) Sterilization	Not destroyed	Destroyed	Destroyed
(b) Soda or Alkali	Not destroyed	Destroyed	Destroyed
(c) Aging	Slowly destroyed	Slowly destroyed	Quickly destroyed
PESERVING			
(a) Tinned meat	Not destroyed	Destroyed	Destroyed
(b) Bottled Vegetables & fruits	Not destroyed	Reduced	Not destroyed
(c) Pickled eggs	Not destroyed	Not destroyed	--
(d) Frozen meat, butter	Not destroyed	Not destroyed	--
(e) Fruit	Not destroyed	Not destroyed	Slowly destroyed
(f) Melting of cereals	Removed	Removed	--
ACID			
(a) Neutral Action	Not destroyed (3)	Highly resistant (4)	Stabilizes somewhat (5)

References for this table are on the following page.

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(1) Plimmer R.H.A. and Plimmer V.G.-"Food, Health, Vitamins" 3rd Edition, Pub. 1928, London, Eng.

(2) Hess A.F. and Unger L.J.- "The Effect of Age, Heat and Reaction on the Antiscorbutic Foods" - Jour. Biol. Chem., Vol. XXXVIII, p. 293, Pub. 1919, Balt., Md., U.S.A.

(3) Voegtlin C. - "The Metabolism of Vitamins" - "Endocrinology and Metabolism"- Vol. 3, p. 341, Pub., 1922, London, Eng.

(4) Sullivan M. and Voegtlin C. - "Distribution in Foods of the so-called Vitamins and their Isolation" - Jour. Biol. Chem., Vol. XXIV, p. 16, 17, Pub. 1916, Balt., Md., U.S.A.

(5) Harden A. and Zilva S.S.- "Differential Behavior of the Antineuritic and Antiscorbutic Factors toward Absorbents"- Jour. Biochem., Vol. XII, p. 93, Pub. 1918, London, Eng.

CHAPTER VIII

Summary

Very little indisputable knowledge is available as to the part played by vitamins in metabolism beyond the fact that the antineuritic and fat-soluble vitamins are needed for growth, that the antisterilic vitamin is needed for reproduction and that all seven vitamins are essential for proper nutrition of the higher animals. Since it is evident that small amounts fulfill the physiological requirements, it is quite possible that vitamins act as catalysts of some metabolic reactions upon nutrition by stimulating the digestive organs to normal function. Considerable work has been done in the past thirty years, in numerous discoveries regarding the physiological and pathological significance of vitamins. Although some facts have been established, this does not hold for all observations made in this field. As a matter of fact, the study of vitamins is still in its infancy, although much work has been done in this field. Further progress will, most likely, depend on chemical isolation of these substances.

One of the most important facts about vitamins is that they cannot be made in the human body and must, therefore, be supplied in the food. They are produced in plants, and vitamins found in animals have come from the plant food. Very few foodstuffs contain all the vitamins; therefore the diet must be a balanced one in vitamin respect.

The vitamins are often referred to in conjunction with the deficiency disease which each one causes, and at one time

were called "vitamine" or "accessory substances". This designation was found not to be a logical one and the vitamins were classified alphabetically and named according to order of discovery (with the exception of vitamin B being classified secondly, yet discovered first by Hopkins).

The greater source of vitamin A is in milk, egg yolk, cod-liver oil and green vegetables. Animals deprived of vitamin A show marked susceptibility to certain kinds of diseases. They develop the characteristic eye disease of ophthalmia or keratomalacia. Animals fed on an A-free-diet show marked retardation of growth which may be an indirect effect of the general malnutrition bringing on A-deficiency; so this vitamin is often called the growth-promoting vitamin. Other diseases are sinus trouble and deafness associated with a deficiency of a vitamin A diet. Chemically, vitamin A is a complex compound of carbon, hydrogen, and oxygen, but, has no nitrogen.

Vitamin B was discovered with a certain kind of neuritis called beri-beri. It is a nitrogen-containing compound belonging to the chemical class known as amines. Any part of the plant contains a fair amount of vitamin B. All fruits and vegetables contain B. The hull layer of wheat kernel has B. Individual foods as yeast, yolk of egg contain it. Lack of vitamin B results in loss of weight and polyneuritis. Varying lack of B results in intermediate action of loss of appetite, constipation, loss of digestion, which lead to diminished vitality.

Two other vitamins are associated with vitamin B. They are vitamins F or B_1, and G or B_2 or PP. Vitamin F or B_1 represents the antineuritic property; the other, B_2 or G or

PP is equally important and is recognized as the antipellagric vitamin. Yeast contains both of these vitamins in considerable abundance. Wheat and corn are poor in the antipellagric potency.

Vitamin C is the scurvy-preventing-antiscorbutic vitamin. This vitamin is both water and alcoholic soluble, whereas vitamin B is soluble in water and in hot ethyl alcohol. Vitamin C is liberally distributed through the vegetable kingdom. The greater source of this vitamin is in the orange, lemon, cabbage, tomato. Milk contains limited amounts; poor as it is in C potency,it can offset scurvy. Defects of teeth are brought on by a vitamin C-deficiency. People depending on salt meat and hard tack have suffered from scurvy, mostly those on long voyages or expeditions.

Vitamin D is fat-soluble, is closely associated with vitamin A in animal fats; yet may or may not be of plant origin. This is the antirachitic vitamin and is necessary for the proper deposition of lime salts in the bones. The chemical substance ergosterol taken in the food can be converted into vitamin D in the body by the action of ultra-violet light. Over-radiation is as negligible as none at all. Vitamin D is abundantly found in cod-liver oil, for fish are exposed to the rays of the sun or eat food that has been exposed to it.

Vitamin E, like vitamin A and D, also occurs in fats, but is present in some vegetable oils which do not contain these other two vitamins. Vitamin E is present in the unsaponifiable portion of the fat. Vitamins A and D and E are not identical, for A is easily destroyed and E is not; further-

more vitamin E is not in that portion of the fat in which one finds vitamin D. Vitamin E is the antisterilic vitamin. If E is lacking in amount, germinal tissues suffer impairment; embryos die in a short time and are reabsorbed into the blood of the mother. This vitamin is found in lettuce and alfalfa, to a certain extent in milk-fat, and most likely in other unstudied foods. Long before vitamins were discovered, a substance necessary as the vitamin is in the animal body for growth, was found to be necessary for growth in the plant. This substance is "Bios" and was found experimentally to be two substances, and is proven to be a chemical compound, inosite.

The inner whitish leaves of vegetables are very poor in vitamin A, while the outer green leaves are richer in this respect. Stalks of bleached vegetables contain no more than traces of vitamin A.

Insects, as the flour moth, require vitamins A and B for normal growth and health just as laboratory animals and human beings do.

Rats do not need vitamin A, D, or C for growth or reproduction but do require a diet containing vitamin E for reproduction. On the other hand, vitamin E is not required for growth. Vitamin B in the diet improves lactation; at present, there is no need for a special vitamin for lactation.

Rats contain on the average a nearly sufficient bodily store of antirachitic vitamin to carry them through a period of 8 weeks, where growth is limited. Irradiation or the feeding of irradiated food to insure an adequate supply of vitamin D

must be taken into account, for the character of the food and the bodily store of vitamin D vary in animals.

Possibly, calcification, as a quantitative test for the presence of vitamin D, is less delicate at the end of a period of 12 weeks on a diet that markedly retards growth than would be a test for calcification at an earlier period. With rats, as experimental subjects, the factor by which femur calcium can be multiplied to obtain total body calcium is in a mean of 56 cases, 14.4.

In considering the factors involved in measuring the relative amounts of vitamin B by the rat growth method, a preliminary depletion period does not increase the accuracy of the work with vitamin B. Larger animals make smaller gains under similar conditions, indication that larger animals have a higher vitamin B requirement. Under like conditions for males and females, it is found that females have a somewhat higher vitamin B requirement. Difference between the average gains for males and females was less than twice its probable error and may possibly have been accidental.

The effect of radiation with the Mercury-Vapour Quartz Lamp on the growth of rats fed on a diet deficient in vitamin A illustrates that normal growth can be prolonged from 35-50 days; without irradiation, growth ceases to be normal after a period of from 7-10 days. Animals cannot be cured of xerophthalmia nor can growth be revived by irradiation after being on an A free-diet for 90 days. Irradiation of rats which had been for shorter periods of 17-35 days on a vitamin-A de-

ficient diet produced a growth response which proved to be inversely proportional, in its duration and extent, to the length of the previous period of deficient diet. Vitamin A is either economized or else the vitamin stores are forced to yield up sufficient A to an extent which suffices to produce normal growth until the supplies are wholly exhausted.

Light can neither create or be substituted for vitamin A; it acts only as an economizer or activator when it is already present.

In preventing rickets by sunlight or ultra-violet light, it is necessary that test animals be on a diet free of vitamin A for a length of time which will prove that protection is permanent.

Experimental evidence does not exclude the possibility that the interplay in growth and rickets is the same; the existence of an interplay in both cases heightens the possibility that the two vitamin factors may be identical or allied.

Vitamins A, B, C are effected differently by heat, chemicals, drying and other processes. Rarely does the effect of an agent on one of these vitamins agree with the effect of the same agent on any of the others. Vitamin D, produced by irradiation of ergosterol, is destroyed by too long irradiation. Ergosterol is a comparatively stable substance. It is not destroyed by heat, chemicals, drying and other processes.

Vitamin B_2 or G or PP is not effected by heat, being very stable in this respect. It is sparingly soluble in alcohol, which is also true of vitamin F.

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